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Sir:

Transmitted herewith for filing under 37 C.F.R. § 1.53(b) is the nonprovisional utility patent application of:

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Enclosed are:

[X] Specification, Claim(s), and Abstract (96 pages).

[X] Informal drawings (29 sheets, Figures 1-16).

[X] Unexecuted Declaration and Power of Attorney (5 pages).

The filing fee is calculated below:

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Title of the Invention

CIRCOVIRUS SEQUENCES ASSOCIATED WITH PIGLET WEIGHT LOSS DISEASE (PWD)

Information on Related Applications

The present application claims the priority benefit, under 35 U.S.C. § 119, of International Application No. PCT/FR98/02634, filed December 4, 1998, which is based upon French Patent Application No. 97 15 396, filed December 5, 1997.

Background of the Invention

The invention relates to the genomic sequence and nucleotide sequences coding for polypeptides of PWD circovirus, such as the structural and nonstructural polypeptides of said circovirus, as well as vectors including said sequences and cells or animals transformed by these vectors. The invention likewise relates to methods for detecting these nucleic acids or polypeptides and kits for diagnosing infection by the PWD circovirus. The invention is also directed to a method for selecting compounds capable of modulating the viral infection. The invention further comprises pharmaceutical compositions, including vaccines, for the prevention and/or the treatment of viral infections by PWD circovirus as well as the use of a vector according to the invention for the prevention and/or the treatment of diseases by gene therapy.

Piglet weight loss disease (PWD), alternatively called fatal piglet wasting (FPW) or Post-Weaning Multisystem Wasting Syndrom (PMWS), has been widely described in North America (Harding, J.C., 1997), and authors have reported the existence of a relationship between this pathology and the presence of porcine circovirus (Daft, B. et al., 1996; Clark, E.G., 1997; Harding, J.C., 1997; Harding, J.C. and Clark, E.G., 1997; Nayar, G.P. et al., 1997). A porcine circovirus has already been demonstrated in established lines of cell cultures derived from pigs and chronically infected (Tischer, I., 1986, 1988, 1995; Dulac, G.C., 1989; Edwards,

S., 1994; Allan, G.M., 1995 and McNeilly, F., 1996). This virus, during experimental infection of piglets, does not prove pathogenic for pigs (Tischer, I., 1986, Horner, G.W., 1991) and its nucleotide sequence has been determined and characterized (Tischer, I., 1982; Meehan, B.M. et al., 1997; Mankertz., A., 1997). The porcine circovirus, called PCV virus, is part of the circovirus genus of the circoviridae family (Murphy, F.A. et al., 1995) whose virion has a circular DNA of size between 1.7 and 2.3 kb, which DNA comprises three open reading frames (ORF1 to ORF3), coding for a replication protein REP involved in the initiation and termination phase of rolling circular replication (RCR) (Heyraud-Nitschke, F., et al., 1995; Harding, M.R. et al., 1993; Hanson, S.F. et al., 1995; Fontes, E.P.B. et al., 1994), coding for a capsid protein (Boulton, L.H. et al., 1997; Hackland, A.F. et al., 1994; Chu, P.W.G. et al., 1993) and coding for a nonstructural protein called a dissemination protein (Lazarowitz., S.G. et al., 1989).

The authors of the present invention have noticed that the clinical signs perceptible in pigs and linked to infection by the PWD circovirus are very distinctive. These manifestations in general appear in pigs of 8 to 12 weeks of age, weaned for 4 to 8 weeks. The first signs are hypotonia without it being possible to speak of prostration. Rapidly (48 hours), the flanks hollow, the line of the spine becomes apparent, and the pigs "blanch." These signs are in general accompanied by hyperthermia, anorexia and most often by respiratory signs (coughing, dyspnea, polypnea). Transitory diarrhea can likewise appear. The disease state phase lasts approximately one month at the end of which the rate of mortality varies from 5 to 20%. To these mortalities, it is expedient to add a variable proportion (5-10%) of cadaveric animals which are no longer able to present an economic future. It is to be noted that outside of this critical stage of the end of post-weaning, no anomaly appears on the farms. In particular, the reproductive function is totally maintained.

On the epidemiological level, the first signs of this pathology appeared at the start of 1995 in the east of the Côtes d'Armor region in France, and the farms affected are especially confined to this area of the region. In December 1996, the

number of farms concerned could not be evaluated with precision because of the absence of a specific laboratory diagnostic method or of an epidemioligical surveillance system of the livestock. Based on the clinical facts as well as on results of postmortem examinations supplied by veterinarians, it is possible to estimate this number as several dozen (80-100). The contagiousness of the disease is weak to moderate. Cases are being reported outside the initial area and for the majority are following the transfer of animals coming from farms familiar with the problem. On the other hand, a characteristic of the condition is its strong remanence. Thus, farms which have been affected for a year are still affected in spite of the massive administration of therapeutics. Farms with clinical expression are drawn from various categories of specialization (breeders/fatteners, post-weaners/ fatteners) and different economic structures are concerned. In addition, the disorders appear even in farms where the rules of animal husbandry are respected.

Numerous postmortem examinations have been carried out either on farms or in the laboratory. The elements of the lesional table are disparate. The most constant macroscopic lesions are pneumonia which sometimes appears in patchy form as well as hypertrophy of the lymphatic ganglia. The other lesions above all affect the thoracic viscera including, especially, pericarditis and pleurisy. However, arthritis and gastric ulcers are also observed. The lesions revealed in the histological examination are essentially situated at the pulmonary level (interstitial pneumonia), ganglionic level (lymphoid depletion of the lymph nodes, giant cells) and renal level (glomerulonephritis, vasculitis). The infectious agents have been the subject of wide research. It has been possible to exclude the intervention of pestiviruses and Aujeszky's disease. The disorders appear in the seropositive PDRS (Porcine Dysgenic and Respiratory Syndrome, an infection linked to an arteriovirus) herds, but it has not been possible to establish the role of the latter in the genesis of the disorders (the majority of the farms in Brittany are PDRS seropositive).

The authors of the present invention, with the aim of identifying the etiological agent responsible for PWD, have carried out "contact" tests between

piglets which are obviously "ill" and SPF pigs (specific pathogen-free) from CNEVA (Centre National d'Etudes Vétérinaires et Alimentaires, France). These tests allow the development of signs comparable to those observed on the farm to be observed in protected animal houses. The discrete signs such as moderate hyperthermia, anorexia and intermittent diarrhea appeared after one week of contact. It must be noted that the PDRS virus only diffused subsequent to the clinical signs. In addition, inocculations of organ homogenates of sick animals to healthy pigs allowed signs related to those observed on the farms to be reproduced, although with a lower incidence, linked to the favorable conditions of upkeep of the animals in the experimental installations.

Thus, the authors of the present invention have been able to demonstrate that the pathological signs appear as a well-defined entity affecting the pig at a particular stage of its growth.

This pathology has never been described in France. However, sparse information, especially Canadian, relates to similar facts.

The disorders cannot be mastered with the existing therapeutics.

The data collected both on the farm and by experimentation have allowed the following points to be higlighted:

- PWD is transmissible but its contagiousness is not very high,
- its etiological origin is of infectious and probably viral nature,
- PWD has a persistent character in the affected farms.

Considerable economic consequences ensue for the farms.

Thus, there is currently a significant need for a specific and sensitive diagnostic, whose production is practical and rapid, allowing the early detection of the infection.

A reliable, sensitive and practical test which allows the distinction between strains of porcine circovirus (PCV) is thus strongly desirable.

On the other hand, a need for efficient and well-tolerated treatment of infections with PWD circovirus likewise remains desirable, no vaccine currently being available against PWD circovirus.

Fuller information concerning the biology of these strains, their interactions with their hosts, the associated infectivity phenomena and those of escape from the immune defenses of the host especially, and finally their implication in the development of associated pathologies, will allow a better understanding of these mechanisms. Taking into account the facts which have been mentioned above and which show in particular the limitations of combatting infection by the PWD circovirus, it is thus essential today on the one hand to develop molecular tools, especially starting from a better genetic knowledge of the PWD circovirus, and likewise to perfect novel preventive and therapeutic treatments, novel methods of diagnosis and specific, efficacious and tolerated novel vaccine strategies. This is precisely the subject of the present invention.

Summary of the Invention

The present invention relates to vaccines comprising a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle. In one embodiment of the invention, the nucleotide sequence is selected from SEQ ID No. 9, SEQ ID No. 10 SEQ ID No. 11, or SEQ ID No. 12, or a homologue or fragment thereof. In another embodiment of the invention, the homologue has at least 80% sequence identity to SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11 or SEQ ID No. 12.. In yet another embodiment, the vaccines further comprising an adjuvant

The present invention also relates to vaccines comprising a polypeptide encoded by a nucleotide sequence of the genome of PCVB, or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle. In one embodiment, the homologue has at least 80% sequence identity to SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11 or SEQ ID No. 12. In another embodiment of the

invention, the nucleotide sequence is selected from SEQ ID No. 11 or SEQ ID No. 12, or a homologue or fragment thereof. In still another embodiment, the polypeptide has the amino acid sequence of SEQ ID No. 14 or SEQ ID No. 15. In yet another embodiment, the homologue has at least 80% sequence identity to SEQ ID No. 14 or SEQ ID No. 15. In another embodiment, the polypeptide has the amino acid sequence of SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, or SEQ ID No. 20.

A further aspect of the invention relates to vaccines comprising a vector and an acceptable pharmaceutical or veterinary vehicle, the vector comprising a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof. In one embodiment, the vaccine further comprises a gene coding for an expression product capable of inhibiting or retarding the establishment or development of a genetic or acquired disease.

The present invention also relates to vaccines comprising a cell and an acceptable pharmaceutical or veterinary vehicle, wherein the cell is transformed with a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof.

Still further, the present invention relates to vaccines comprising a pharmaceutically acceptable vehicle and a single polypetide, wherein the single polypeptide consists of SEQ ID No. 15.

Additionally, the present invention relates to methods of immunizing a mammal against piglet weight loss disease comprising administering to a mammal an effective amount of the vaccines desribed above.

These and other aspects of the invention will become apparent to the skilled artisan in view of the teachings contained herein.

Brief Description of the Drawings

Figure 1: Experimental scheme which has made it possible to bring about the isolation and the identification of the circovirus associated with PWD of type A and B.

Test 1: experimental reproduction of the PWD by inoculation of pig organ homogenates from farms affected by PWD.

Test 2: experimental reproduction of PWD.

Test 3: experimental reproduction of PWD.

Test 4: no experimental reproduction of PWD.

Figure 2: Organization of the genome of the circovirus associated with PWD of type A (PCVA)

- strand of (+) polarity (SEQ ID No. 1);
- strand of (-) polarity (SEQ ID No. 2, represented according to the orientation 3' → 5');
- sequences of amino acids of proteins encoded by the two DNA strands in the three possible reading frames.

Figure 3: Alignment of the nucleotide sequence SEQ ID No. 1 of the PWD circovirus of type A (PCVA) and of the MEEHAN strain and MANKERTZ strain circoviruses of the porcine cell lines.

Figure 4: Alignment of the sequence of amino acids SEQ ID No. 6 of a polypeptide encoded by the nucleotide sequence SEQ ID No. 3 (ORF1) of the PWD circovirus of type A (PCVA) and of corresponding nucleotide sequences of the MEEHAN strain and MANKERTZ strain circoviruses of the porcine cell lines.

Figure 5: Alignment of the sequence of amino acids SEQ ID No. 7 of a polypeptide encoded by the nucleotide sequence SEQ ID No. 4 (ORF2) of the PWD

circovirus of type A (PCVA) and of corresponding nucleotide sequences of the MEEHAN strain and MANKERTZ strain circoviruses of the porcine cell lines.

Figure 6: Alignment of the sequence of amino acids SEQ ID No. 8 of a polypeptide encoded by the nucleotide sequence SEQ ID No. 5 (ORF3) of the PWD circovirus of type A (PCVA) and of corresponding nucleotide sequences of the MEEHAN strain and MANKERTZ strain circoviruses of the porcine cell lines.

Figure 7: Western blot analysis of recombinant proteins of the PWD circovirus of type A (PCVA).

The analyses were carried out on cell extracts of Sf9 cells obtained after infection with recombinant baculovirus PCF ORF 1.

Figure 8: Organization of the genome of the circovirus associated with the PWD of type B (PCVB)

- strand of (+) polarity (SEQ ID No. 9);
- strand of (-) polarity (SEQ ID No. 10, represented according to the orientation 3' → 5');
- sequence of amino acids of proteins encoded by the two DNA strands in the three possible reading frames.

Figure 9: Evolution of the daily mean gain (DMG) of pig farms affected by piglet weight loss disease (PWD), placed under experimental conditions.

Figure 10: DMG compared for the 3 batches of pigs (F1, F3 and F4) calculated over a period of 28 days, after vaccination test.

Figure 11: Hyperthermia greater than 41°C, expressed as a percentage compared for the 3 batches of pigs (F1, F3 and F4) calculated per week over a period of 28 days, after vaccination test.

Figure 12: Membranes of peptide spots corresponding to the ORF2s revealed with the aid of an infected pig serum, originating from a conventional farm.

The numbers of specific peptides of the circovirus of type B as well as their nonreactive homologs (type A) are indicated in bold.

The nonspecific immunogenic peptides are indicated in italics.

Figure 13: Alignment of amino acid sequences of proteins encoded by the ORF2 of the PWD circovirus of type A and by the ORF'2 of the PWD circovirus of type B. The position of 4 peptides corresponding to specific epitopes of the PWD circovirus of type B is indicated on the corresponding sequence by a bold line, their homolog on the sequence of the PWD circovirus of type A is likewise indicated by an ordinary line.

Figure 14: Charts the results of experiments that demonstrate, in terms of percent hyperthermia, that vaccination with ORF'1 and ORF'2 of PCV-B enhances the level of protection in swine challeneged with PCV-B.

Figure 15: Charts the results of experiments that demonstrate, in terms of animal growth, that vaccination with ORF'1 and ORF'2 of PCV-B enhances the level of protection in swine challeneged with PCV-B.

Figure 16: Immunoperoxidase staining of PK15 cells at 24 h post-transfection with the pcDNA3/ORF'2 plasmid. Expression of PCVB ORF'2 was

confirmed by IPMA following incubation in the presence of the swine anti-PCVB monospecific serum

Detailed Description of the Invention

The present invention relates to nucleotide sequences of the genome of PWD circovirus selected from the sequences SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 9, SEQ ID No. 10 or one of their fragments.

The nucleotide sequences of sequences SEQ ID No. 1 and SEQ ID No. 2 correspond respectively to the genome sequence of the strand of (+) polarity and of the strand of (-) polarity of the PWD circovirus of type A (or PCVA), the sequence SEQ ID No. 2 being represented according to the orientation 5'→3'.

The nucleotide sequences of sequences SEQ ID No. 9 and SEQ ID No. 10 correspond respectively to the genome sequence of the strand of (+) polarity and of the strand of (-) polarity of the PWD circovirus of type B (or PCVB), the sequence SEQ ID No. 10 being represented according to the orientation 5'→3'.

The present invention likewise relates to nucleotide sequences, characterized in that they are selected from:

a) a nucleotide sequence of a specific fragment of the sequence SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 9, SEQ ID No. 10 or one of their fragments;

b) a nucleotide sequence homologous to a nucleotide sequence such as defined in a);

c) a nucleotide sequence complementary to a nucleotide sequence such as defined in a) or b), and a nucleotide sequence of their corresponding RNA;

d) a nucleotide sequence capable of hybridizing under stringent conditions with a sequence such as defined in a), b) or c);

e) a nucleotide sequence comprising a sequence such as defined in a), b), c) or d); and

f) a nucleotide sequence modified by a nucleotide sequence such as defined in a), b), c), d) or e).

Nucleotide, polynucleotide or nucleic acid sequence will be understood according to the present invention as meaning both a double-stranded or single-stranded DNA in the monomeric and dimeric (so-called in tandem) forms and the transcription products of said DNAs.

It must be understood that the present invention does not relate to the genomic nucleotide sequences taken in their natural environment, that is to say in the natural state. It concerns sequences which it has been possible to isolate, purify or partially purify, starting from separation methods such as, for example, ion-exchange chromatography, by exclusion based on molecular size, or by affinity, or alternatively fractionation techniques based on solubility in different solvents, or starting from methods of genetic engineering such as amplification, cloning and subcloning, it being possible for the sequences of the invention to be carried by vectors.

The nucleotide sequences SEQ ID No. 1 and SEQ ID No. 9 were obtained by sequencing of the genome by the Sanger method.

Nucleotide sequence fragment according to the invention will be understood as designating any nucleotide fragment of the PWD circovirus, type A or B, of length of at least 8 nucleotides, preferably at least 12 nucleotides, and even more preferentially at least 20 consecutive nucleotides of the sequence from which it originates.

Specific fragment of a nucleotide sequence according to the invention will be understood as designating any nucleotide fragment of the PWD circovirus, type A or B, having, after alignment and comparison with the corresponding fragments of known porcine circoviruses, at least one nucleotide or base of different nature. For example, the specific nucleotide fragments of the PWD circovirus of type A can easily be determined by referring to Figure 3 of the present invention in which the nucleotides or bases of the sequence SEQ ID No. 1 (circopordfp) are shown which are of different nature, after alignment of said sequence SEQ ID No. 1 with the other two sequences of known porcine circovirus (circopormeeh and circopormank).

Homologous nucleotide sequence in the sense of the present invention is understood as meaning a nucleotide sequence having at least a percentage identity with the bases of a nucleotide sequence according to the invention of at least 80%, preferably 90% or 95%, this percentage being purely statistical and it being possible to distribute the differences between the two nucleotide sequences at random and over the whole of their length.

Specific homologous nucleotide sequence in the sense of the present invention is understood as meaning a homologous nucleotide sequence having at least one nucleotide sequence of a specific fragment, such as defined above. Said "specific" homologous sequences can comprise, for example, the sequences corresponding to the genomic sequence or to the sequences of its fragments representative of variants of PWD circovirus of type A or B. These specific homologous sequences can thus correspond to variations linked to mutations within strains of PWD circovirus of type A and B, and especially correspond to truncations, substitutions, deletions and/or additions of at least one nucleotide. Said homologous sequences can likewise correspond to variations linked to the degeneracy of the genetic code.

The term "degree or percentage of sequence homology" refers to "degree or percentage of sequence identity between two sequences after optimal alignment" as defined in the present application.

Two amino-acids or nucleotidic sequences are said to be "identical" if the sequence of amino-acids or nucleotidic residues, in the two sequences is the same when aligned for maximum correspondence as described below. Sequence comparisons between two (or more) peptides or polynucleotides are typically performed by comparing sequences of two optimally aligned sequences over a segment or "comparison window" to identify and compare local regions of sequence similarity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, *Ad. App. Math* 2: 482 (1981), by the homology alignment algorithm of Neddleman and Wunsch, J. *Mol. Biol.* 48:

443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci.* (U.S.A.) 85: 2444 (1988), by computerized implementation of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by visual inspection.

"Percentage of sequence identity" (or degree or identity) is determined by comparing two optimally aligned sequences over a comparison window, where the portion of the peptide or polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino-acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

The definition of sequence identity given above is the definition that would use one of skill in the art. The definition by itself does not need the help of any algorithm, said algorithms being helpful only to achieve the optimal alignments of sequences, rather than the calculation of sequence identity.

From the definition given above, it follows that there is a well defined and only one value for the sequence identity between two compared sequences which value corresponds to the value obtained for the best or optimal alignment.

In the BLAST N or BLAST P "BLAST 2 sequence", software which is available in the web site http://www.ncbi.nlm.nih.gov/gorf/bl2.html, and habitually used by the inventors and in general by the skilled man for comparing and determining the identity between two sequences, gap cost which depends on the sequence length to be compared is directly selected by the software (i.e. 11.2 for substitution matrix BLOSUM-62 for length > 85).

In the present description, PWD circovirus will be understood as designating the circoviruses associated with piglet weight loss disease (PWD) of type A (PCVA) or type B (PCVB), defined below by their genomic sequence, as well as the circoviruses whose nucleic sequences are homologous to the sequences of PWD circoviruses of type A or B, such as in particular the circoviruses corresponding to variants of the type A or of the type B.

Complementary nucleotide sequence of a sequence of the invention is understood as meaning any DNA whose nucleotides are complementary to those of the sequence of the invention, and whose orientation is reversed (antiparallel sequence).

Hybridization under conditions of stringency with a nucleotide sequence according to the invention is understood as meaning a hybridization under conditions of temperature and ionic strength chosen in such a way that they allow the maintenance of the hybridization between two fragments of complementary DNA.

By way of illustration, conditions of great stringency of the hybridization step with the aim of defining the nucleotide fragments described above are advantageously the following.

The hybridization is carried out at a preferential temperature of 65°C in the presence of SSC buffer, 1 × SSC corresponding to 0.15 M NaCl and 0.05 M Na citrate. The washing steps, for example, can be the following:

- 2 × SSC, at ambient temperature followed by two washes with 2 × SSC, 0.5% SDS at 65°C; 2 × 0.5 × SSC, 0.5% SDS; at 65°C for 10 minutes each.

The conditions of intermediate stringency, using, for example, a temperature of 42°C in the presence of a 2 × SSC buffer, or of less stringency, for example a temperature of 37°C in the presence of a 2 × SSC buffer, respectively require a globally less significant complementarity for the hybridization between the two sequences.

The stringent hybridization conditions described above for a polynucleotide with a size of approximately 350 bases will be adapted by the person skilled in the art for oligonucleotides of greater or smaller size, according to the teaching of Sambrook et al., 1989.

Among the nucleotide sequences according to the invention, those are likewise preferred which can be used as a primer or probe in methods allowing the homologous sequences according to the invention to be obtained, these methods, such as the polymerase chain reaction (PCR), nucleic acid cloning and sequencing, being well known to the person skilled in the art.

Among said nucleotide sequences according to the invention, those are again preferred which can be used as a primer or probe in methods allowing the presence of PWD circovirus or one of its variants such as defined below to be diagnosed.

The nucleotide sequences according to the invention capable of modulating, of inhibiting or of inducing the expression of PWD circovirus gene, and/or capable of modulating the replication cycle of PWD circovirus in the host cell and/or organism are likewise preferred. Replication cycle will be understood as designating the invasion and the multiplication of PWD circovirus, and its propagation from host cell to host cell in the host organism.

Among said nucleotide sequences according to the invention, those corresponding to open reading frames, called ORF sequences, and coding for polypeptides, such as, for example, the sequences SEQ ID No. 3 (ORF1), SEQ ID No. 4 (ORF2) and SEQ ID No. 5 (ORF3) respectively corresponding to the nucleotide sequences between the positions 47 and 985 determined with respect to the position of the nucleotides on the sequence SEQ ID No. 1, the positions 1723 and 1022 and the positions 658 and 38 with respect to the position of the nucleotides on the sequence SEQ ID No. 2 (represented according to the orientation 3'→5'), the ends being included, or alternatively the sequences SEQ ID No. 11 (ORF'1), SEQ ID No. 12 (ORF'2) and SEQ ID No. 13 (ORF'3), respectively corresponding to the sequences between the positions 51 and 995 determined with respect to the position

of the nucleotides on the sequence SEQ ID No. 9, the positions 1734 and 1033 and the positions 670 and 357, the positions being determined with respect to the position of the nucleotides on the sequence SEQ ID No. 10 (represented according to the orientation 3'→5'), the ends being included, are finally preferred.

The nucleotide sequence fragments according to the invention can be obtained, for example, by specific amplification, such as PCR, or after digestion with appropriate restriction enzymes of nucleotide sequences according to the invention, these methods in particular being described in the work of Sambrook et al., 1989. Said representative fragments can likewise be obtained by chemical synthesis when their size is not very large and according to methods well known to persons skilled in the art.

Modified nucleotide sequence will be understood as meaning any nucleotide sequence obtained by mutagenesis according to techniques well known to the person skilled in the art, and containing modifications with respect to the normal sequences according to the invention, for example mutations in the regulatory and/or promoter sequences of polypeptide expression, especially leading to a modification of the rate of expression of said polypeptide or to a modulation of the replicative cycle.

Modified nucleotide sequence will likewise be understood as meaning any nucleotide sequence coding for a modified polypeptide such as defined below.

The present invention relates to nucleotide sequences of PWD circovirus according to the invention, characterized in that they are selected from the sequences SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13 or one of their fragments.

The invention likewise relates to nucleotide sequences characterized in that they comprise a nucleotide sequence selected from:

a) a nucleotide sequence SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13 or one of their fragments;

b) a nucleotide sequence of a specific fragment of a sequence such as defined in a);

c) a homologous nucleotide sequence having at least 80% identity with a sequence such as defined in a) or b);

d) a complementary nucleotide sequence or sequence of RNA corresponding to a sequence such as defined in a), b) or c); and

e) a nucleotide sequence modified by a sequence such as defined in a), b), c) or d).

As far as homology with the nucleotide sequences SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13 or one of their fragments is concerned, the homologous, especially specific, sequences having a percentage identity with one of the sequences SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13 or one of their fragments of at least 80%, preferably 90% or 95%, are preferred. Said specific homologous sequences can comprise, for example, the sequences corresponding to the sequences ORF1, ORF2, ORF3, ORF'1, ORF'2 and ORF'3 of PWD circovirus variants of type A or of type B. In the same manner, these specific homologous sequences can correspond to variations linked to mutations within strains of PWD circovirus of type A or of type B and especially correspond to truncations, substitutions, deletions and/or additions of at least one nucleotide.

Among nucleotide sequences according to the invention, the sequence SEQ ID No. 11 which has a homology having more than 80% identity with the sequence SEQ ID No. 3, as well as the sequence SEQ ID No. 12, are especially preferred.

Preferably, the invention relates to the nucleotide sequences according to the invention, characterized in that they comprise a nucleotide sequence selected from the following sequences:

a) 170 5' TGTGGCGA 3';

b) 450 5' AGTTTCCT 3';

c) 1026 5' TCATTTAGAGGGTCTTTCAG 3';

d) 1074 5' GTCAACCT 3';

e) 1101 5' GTGGTTGC 3';

f) 1123 5' AGCCCAGG 3';

g) 1192 5' TTGGCTGG 3';

h) 1218 5' TCTAGCTCTGGT 3';

i) 1501 5' ATCTCAGCTCGT 3';

j) 1536 5' TGTCCTCCTCTT 3';

k) 1563 5' TCTCTAGA 3';

l) 1623 5' TGTACCAA 3';

m) 1686 5' TCCGTCTT 3';

and their complementary sequences.

In the list of nucleotide sequences a)-m) above, the underlined nucleotides are mutated with respect to the two known sequences of circovirus which are nonpathogenic to pigs. The number preceding the nucleotide sequence represents the position of the first nucleotide of said sequence in the sequence SEQ ID No. 1.

The invention comprises the polypeptides encoded by a nucleotide sequence according to the invention, preferably a polypeptide whose sequence is represented by a fragment, especially a specific fragment, of one of the six sequences of amino acids represented in Figure 2, these six amino acid sequences corresponding to the polypeptides which can be encoded according to one of the three possible reading frames of the sequence SEQ ID No. 1 or of the sequence SEQ ID No. 2, or a polypeptide whose sequence is represented by a fragment, especially a specific fragment, of one of the six sequences of amino acids shown in Figure 8, these six sequences of amino acids corresponding to the polypeptides which can be encoded according to one of the three possible reading frames of the sequence SEQ ID No. 9 or of the sequence SEQ ID No. 10.

The invention likewise relates to the polypeptides, characterized in that they comprise a polypeptide selected from the amino acid sequences SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 14, SEQ ID No. 15, SEQ ID No. 16 or one of their fragments.

Among the polypeptides according to the invention, the polypeptide of amino acid sequence SEQ ID No. 14 which has a homology having more than 80% identity with the sequence SEQ ID No. 6, as well as the polypeptide of sequence SEQ ID No. 15, are especially preferred.

The invention also relates to the polypeptides, characterized in that they comprise a polypeptide selected from:

a) a specific fragment of at least 5 amino acids of a polypeptide of an amino acid sequence according to the invention;

b) a polypeptide homologous to a polypeptide such as defined in a);

c) a specific biologically active fragment of a polypeptide such as defined in a) or b); and

d) a polypeptide modified by a polypeptide such as defined in a), b) or c).

Among the polypeptides according to the invention, the polypeptides of amino acid sequences SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19 and SEQ ID No. 20 are also preferred, these polypeptides being especially capable of specifically recognizing the antibodies produced during infection by the PWD circovirus of type B. These polypeptides thus have epitopes specific for the PWD circovirus of type B and can thus be used in particular in the diagnostic field or as immunogenic agent to confer protection in pigs against infection by PWD circovirus, especially of type B.

In the present description, the terms polypeptide, peptide and protein are interchangeable.

It must be understood that the invention does not relate to the polypeptides in natural form, that is to say that they are not taken in their natural environment but that they can be isolated or obtained by purification from natural sources, or else obtained by genetic recombination, or alternatively by chemical synthesis and that they can thus contain unnatural amino acids, as will be described below.

Polypeptide fragment according to the invention is understood as designating a polypeptide containing at least 5 consecutive amino acids, preferably 10 consecutive amino acids or 15 consecutive amino acids.

In the present invention, specific polypeptide fragment is understood as designating the consecutive polypeptide fragment encoded by a specific fragment nucleotide sequence according to the invention.

Homologous polypeptide will be understood as designating the polypeptides having, with respect to the natural polypeptide, certain modifications such as, in particular, a deletion, addition or substitution of at least one amino acid, a truncation, a prolongation, a chimeric fusion, and/or a mutation. Among the homologous polypeptides, those are preferred whose amino acid sequence has at least 80%, preferably 90%, homology with the sequences of amino acids of polypeptides according to the invention.

Specific homologous polypeptide will be understood as designating the homologous polypeptides such as defined above and having a specific fragment of polypeptide according to the invention.

In the case of a substitution, one or more consecutive or nonconsecutive amino acids are replaced by "equivalent" amino acids. The expression "equivalent" amino acid is directed here at designating any amino acid capable of being substituted by one of the amino acids of the base structure without, however, essentially modifying the biological activities of the corresponding peptides and such that they will be defined by the following.

These equivalent amino acids can be determined either by depending on their structural homology with the amino acids which they substitute, or on results of comparative tests of biological activity between the different polypeptides, which are capable of being carried out.

By way of example, the possibilities of substitutions capable of being carried out without resulting in an extensive modification of the biological activity of the corresponding modified polypeptides will be mentioned, the replacement, for

example, of leucine by valine or isoleucine, of aspartic acid by glutamic acid, of glutamine by asparagine, of arginine by lysine etc., the reverse substitutions naturally being envisageable under the same conditions.

The specific homologous polypeptides likewise correspond to polypeptides encoded by the specific homologous nucleotide sequences such as defined above and thus comprise in the present definition the polypeptides which are mutated or correspond to variants which can exist in PWD circovirus, and which especially correspond to truncations, substitutions, deletions and/or additions of at least one amino acid residue.

Specific biologically active fragment of a polypeptide according to the invention will be understood in particular as designating a specific polypeptide fragment, such as defined above, having at least one of the characteristics of polypeptides according to the invention, especially in that it is:

- capable of inducing an immunogenic reaction directed against a PWD circovirus; and/or
- capable of being recognized by a specific antibody of a polypeptide according to the invention; and/or
- capable of linking to a polypeptide or to a nucleotide sequence of PWD circovirus; and/or
- capable of exerting a physiological activity, even partial, such as, for example, a dissemination or structural (capsid) activity; and/or
- capable of modulating, of inducing or of inhibiting the expression of PWD circovirus gene or one of its variants, and/or capable of modulating the replication cycle of PWD circovirus in the cell and/or the host organism.

The polypeptide fragments according to the invention can correspond to isolated or purified fragments naturally present in a PWD circovirus or correspond to fragments which can be obtained by cleavage of said polypeptide by a proteolytic enzyme, such as trypsin or chymotrypsin or collagenase, or by a chemical reagent, such as cyanogen bromide (CNBr) or alternatively by placing said polypeptide in a

very acidic environment, for example at pH 2.5. Such polypeptide fragments can likewise just as easily be prepared by chemical synthesis, from hosts transformed by an expression vector according to the invention containing a nucleic acid allowing the expression of said fragments, placed under the control of appropriate regulation and/or expression elements.

"Modified polypeptide" of a polypeptide according to the invention is understood as designating a polypeptide obtained by genetic recombination or by chemical synthesis as will be described below, having at least one modification with respect to the normal sequence. These modifications will especially be able to bear on amino acids at the origin of a specificity, of pathogenicity and/or of virulence, or at the origin of the structural conformation, and of the capacity of membrane insertion of the polypeptide according to the invention. It will thus be possible to create polypeptides of equivalent, increased or decreased activity, and of equivalent, narrower, or wider specificity. Among the modified polypeptides, it is necessary to mention the polypeptides in which up to 5 amino acids can be modified, truncated at the N- or C-terminal end, or even deleted or added.

As is indicated, the modifications of the polypeptide will especially have as objective:

- to render it capable of modulating, of inhibiting or of inducing the expression of PWD circovirus gene and/or capable of modulating the replication cycle of PWD circovirus in the cell and/or the host organism,
- of allowing its incorporation into vaccine compositions,
- of modifying its bioavailability as a compound for therapeutic use.

The methods allowing said modulations on eukaryotic or prokaryotic cells to be demonstrated are well known to the person skilled in the art. It is likewise well understood that it will be possible to use the nucleotide sequences coding for said modified polypeptides for said modulations, for example through vectors according to the invention and described below, in order, for example, to prevent or to treat the pathologies linked to the infection.

The preceding modified polypeptides can be obtained by using combinatorial chemistry, in which it is possible to systematically vary parts of the polypeptide before testing them on models, cell cultures or microorganisms for example, to select the compounds which are most active or have the properties sought.

Chemical synthesis likewise has the advantage of being able to use:

- unnatural amino acids, or
- nonpeptide bonds.

Thus, in order to improve the duration of life of the polypeptides according to the invention, it may be of interest to use unnatural amino acids, for example in D form, or else amino acid analogs, especially sulfur-containing forms, for example.

Finally, it will be possible to integrate the structure of the polypeptides according to the invention, its specific or modified homologous forms, into chemical structures of polypeptide type or others. Thus, it may be of interest to provide at the N- and C-terminal ends compounds not recognized by the proteases.

The nucleotide sequences coding for a polypeptide according to the invention are likewise part of the invention.

The invention likewise relates to nucleotide sequences utilizable as a primer or probe, characterized in that said sequences are selected from the nucleotide sequences according to the invention.

Among the pairs of nucleotide sequences utilizable as a pair of primers according to the invention, the pairs of primers selected from the following pairs are preferred:

a) 5' GTG TGC TCG ACA TTG GTG TG 3', and
5' TGG AAT GTT AAC GAG CTG AG 3';

b) 5' GTG TGC TCG ACA TTG GTG TG 3', and
5' CTC GCA GCC ATC TTG GAA TG 3';

c) 5' CGC GCG TAA TAC GAC TCA CT 3', and
5' GTG TGC TCG ACA TTG GTG TG 3';

d) 5' CGC GCG TAA TAC GAC TCA CT 3', and
5' CTC GCA GCC ATC TTG GAA TG 3'; and

e) 5' CCT GTC TAC TGC TGT GAG TAC CTT GT 3', and
5' GCA GTA GAC AGG TCA CTC CGT TGT CC 3'.

The cloning and the sequencing of the PWD circovirus, type A and B, has allowed it to be identified, after comparative analysis with the nucleotide sequences of other porcine circoviruses, that, among the sequences of fragments of these nucleic acids, were those which are strictly specific to the PWD circovirus of type A, of type B or of type A and B, and those which correspond to a consensus sequence of porcine circoviruses other than the PWD circoviruses of type A and/or B.

There is likewise a great need for nucleotide sequences utilizable as a primer or probe specific to the whole of the other known and nonpathogenic porcine circoviruses.

Said consensus nucleotide sequences specific to all circoviruses, other than PWD circovirus of type A and B, are easily identifiable from Figure 3 and the sequence SEQ ID No. 9, and are part of the invention.

Among said consensus nucleotide sequences, that which is characterized in that it is part of the following pair of primers is preferred:

a) 5' GTG TGC TCG ACA TTG GTG TG 3', and
5' TGG AAT GTT AAC TAC CTC AA 3'.

The invention likewise comprises a nucleotide sequence according to the invention, characterized in that said sequence is a specific consensus sequence of porcine circovirus other than PWD circovirus of type B and in that it is one of the primers of the following pairs of primers:

a) 5' GGC GGC GCC ATC TGT AAC GGT TT 3', and
5' GAT GGC GCC GAA AGA CGG GTA TC 3'.

It is well understood that the present invention likewise relates to specific polypeptides of known porcine circoviruses other than PWD circovirus, encoded by

said consensus nucleotide sequences, capable of being obtained by purification from natural polypeptides, by genetic recombination or by chemical synthesis by procedures well known to the person skilled in the art and such as described in particular below. In the same manner, the labeled or unlabeled mono- or polyclonal antibodies directed against said specific polypeptides encoded by said consensus nucleotide sequences are also part of the invention.

It will be possible to use said consensus nucleotide sequences, said corresponding polypeptides as well as said antibodies directed against said polypeptides in procedures or sets for detection and/or identification such as described below, in place of or in addition to nucleotide sequences, polypeptides or antibodies according to the invention, specific to PWD circovirus type A and/or B.

These protocols have been improved for the differential detection of the circular monomeric forms of specific replicative forms of the virion or of the DNA in replication and the dimeric forms found in so-called in-tandem molecular constructs.

The invention additionally relates to the use of a nucleotide sequence according to the invention as a primer or probe for the detection and/or the amplification of nucleic acid sequences.

The nucleotide sequences according to the invention can thus be used to amplify nucleotide sequences, especially by the PCR technique (polymerase chain reaction) (Erlich, 1989; Innis et al., 1990; Rolfs et al., 1991; and White et al., 1997).

These oligodeoxyribonucleotide or oligoribonucleotide primers advantageously have a length of at least 8 nucleotides, preferably of at least 12 nucleotides, and even more preferentially at least 20 nucleotides.

Other amplification techniques of the target nucleic acid can be advantageously employed as alternatives to PCR.

The nucleotide sequences of the invention, in particular the primers according to the invention, can likewise be employed in other procedures of amplification of a target nucleic acid, such as:

- the TAS technique (Transcription-based Amplification System), described by Kwoh et al. in 1989;
- the 3SR technique (Self-Sustained Sequence Replication), described by Guatelli et al. in 1990;
- the NASBA technique (Nucleic Acid Sequence Based Amplification), described by Kievitis et al. in 1991;
- the SDA technique (Strand Displacement Amplification) (Walker et al., 1992);
- the TMA technique (Transcription Mediated Amplification).

The polynucleotides of the invention can also be employed in techniques of amplification or of modification of the nucleic acid serving as a probe, such as:

- the LCR technique (Ligase Chain Reaction), described by Landegren et al. in 1988 and improved by Barany et al. in 1991, which employs a thermostable ligase;
- the RCR technique (Repair Chain Reaction), described by Segev in 1992;
- the CPR technique (Cycling Probe Reaction), described by Duck et al. in 1990;
- the amplification technique with Q-beta replicase, described by Miele et al. in 1983 and especially improved by Chu et al. in 1986, Lizardi et al. in 1988, then by Burg et al. as well as by Stone et al. in 1996.

In the case where the target polynucleotide to be detected is possibly an RNA, for example an mRNA, it will be possible to use, prior to the employment of an amplification reaction with the aid of at least one primer according to the invention or to the employment of a detection procedure with the aid of at least one probe of the invention, an enzyme of reverse transcriptase type in order to obtain a cDNA from the RNA contained in the biological sample. The cDNA obtained will

thus serve as a target for the primer(s) or the probe(s) employed in the amplification or detection procedure according to the invention.

The detection probe will be chosen in such a manner that it hybridizes with the target sequence or the amplicon generated from the target sequence. By way of sequence, such a probe will advantageously have a sequence of at least 12 nucleotides, in particular of at least 20 nucleotides, and preferably of at least 100 nucleotides.

The invention also comprises the nucleotide sequences utilizable as a probe or primer according to the invention, characterized in that they are labeled with a radioactive compound or with a nonradioactive compound.

The unlabeled nucleotide sequences can be used directly as probes or primers, although the sequences are generally labeled with a radioactive element (^{32}P, ^{35}S, ^{3}H, ^{125}I) or with a nonradioactive molecule (biotin, acetylaminofluorene, digoxigenin, 5-bromodeoxyuridine, fluorescein) to obtain probes which are utilizable for numerous applications.

Examples of nonradioactive labeling of nucleotide sequences are described, for example, in French Patent No. 78.10975 or by Urdea et al. or by Sanchez-Pescador et al. in 1988.

In the latter case, it will also be possible to use one of the labeling methods described in patents FR-2 422 956 and FR-2 518 755.

The hybridization technique can be carried out in various manners (Matthews et al., 1988). The most general method consists in immobilizing the nucleic acid extract of cells on a support (such as nitrocellulose, nylon, polystyrene) and in incubating, under well-defined conditions, the immobilized target nucleic acid with the probe. After hybridization, the excess of probe is eliminated and the hybrid molecules formed are detected by the appropriate method (measurement of the radioactivity, of the fluorescence or of the enzymatic activity linked to the probe).

The invention likewise comprises the nucleotide sequences according to the invention, characterized in that they are immobilized on a support, covalently or noncovalently.

According to another advantageous mode of employing nucleotide sequences according to the invention, the latter can be used immobilized on a support and can thus serve to capture, by specific hybridization, the target nucleic acid obtained from the biological sample to be tested. If necessary, the solid support is separated from the sample and the hybridization complex formed between said capture probe and the target nucleic acid is then detected with the aid of a second probe, a so-called detection probe, labeled with an easily detectable element.

Another subject of the present invention is a vector for the cloning and/or expression of a sequence, characterized in that it contains a nucleotide sequence according to the invention.

The vectors according to the invention, characterized in that they contain the elements allowing the expression and/or the secretion of said nucleotide sequences in a determined host cell, are likewise part of the invention.

The vector must then contain a promoter, signals of initiation and termination of translation, as well as appropriate regions of regulation of transcription. It must be able to be maintained stably in the host cell and can optionally have particular signals specifying the secretion of the translated protein. These different elements are chosen as a function of the host cell used. To this end, the nucleotide sequences according to the invention can be inserted into autonomous replication vectors within the chosen host, or integrated vectors of the chosen host.

Such vectors will be prepared according to the methods currently used by the person skilled in the art, and it will be possible to introduce the clones resulting therefrom into an appropriate host by standard methods, such as, for example, lipofection, electroporation and thermal shock.

The vectors according to the invention are, for example, vectors of plasmid or viral origin.

A preferred vector for the expression of polypeptides of the invention is baculovirus.

The vector pBS KS in which is inserted the in-tandem DNA sequence of the PWD circovirus type A (or DFP) as deposited at the CNCM on 3 July 1997, under the number I-1891, is likewise preferred.

These vectors are useful for transforming host cells in order to clone or to express the nucleotide sequences of the invention.

The invention likewise comprises the host cells transformed by a vector according to the invention.

These cells can be obtained by the introduction into host cells of a nucleotide sequence inserted into a vector such as defined above, then the culturing of said cells under conditions allowing the replication and/or expression of the transfected nucleotide sequence.

The host cell can be selected from prokaryotic or eukaryotic systems, such as, for example, bacterial cells (Olins and Lee, 1993), but likewise yeast cells (Buckholz, 1993), as well as animal cells, in particular the cultures of mammalian cells (Edwards and Aruffo, 1993), and especially Chinese hamster ovary (CHO) cells, but likewise the cells of insects in which it is possible to use procedures employing baculoviruses, for example (Luckow, 1993).

A preferred host cell for the expression of the proteins of the invention is constituted by sf9 insect cells.

A more preferred host cell according to the invention is E. coli, such as deposited at the CNCM on 3 July 1997, under the number I-1891.

The invention likewise relates to animals comprising one of said transformed cells according to the invention.

The obtainment of transgenic animals according to the invention overexpressing one or more of the genes of PWD circovirus or part of the genes will be preferably carried out in rats, mice or rabbits according to methods well known to the person skilled in the art, such as by viral or nonviral transfections. It

will be possible to obtain the transgenic animals overexpressing one or more of said genes by transfection of multiple copies of said genes under the control of a strong promoter of ubiquitous nature, or selective for one type of tissue. It will likewise be possible to obtain the transgenic animals by homologous recombination in embryonic cell strains, transfer of these cell strains to embryos, selection of the affected chimeras at the level of the reproductive lines, and growth of said chimeras.

The transformed cells as well as the transgenic animals according to the invention are utilizable in procedures for preparation of recombinant polypeptides.

It is today possible to produce recombinant polypeptides in relatively large quantity by genetic engineering using the cells transformed by expression vectors according to the invention or using transgenic animals according to the invention.

The procedures for preparation of a polypeptide of the invention in recombinant form, characterized in that they employ a vector and/or a cell transformed by a vector according to the invention and/or a transgenic animal comprising one of said transformed cells according to the invention, are themselves comprised in the present invention.

Among said procedures for preparation of a polypeptide of the invention in recombinant form, the preparation procedures employing a vector, and/or a cell transformed by said vector and/or a transgenic animal comprising one of said transformed cells, containing a nucleotide sequence according to the invention coding for a polypeptide of PWD circovirus, are preferred.

The recombinant polypeptides obtained as indicated above can just as well be present in glycosylated form as in nonglycosylated form and can or cannot have the natural tertiary structure.

A preferred variant consists in producing a recombinant polypeptide used to a "carrier" protein (chimeric protein). The advantage of this system is that it allows a stabilization of and a decrease in the proteolysis of the recombinant product, an increase in the solubility in the course of renaturation in vitro and/or a simplification of the purification when the fusion partner has an affinity for a specific ligand.

More particularly, the invention relates to a procedure for preparation of a polypeptide of the invention comprising the following steps:

a) culture of transformed cells under conditions allowing the expression of a recombinant polypeptide of nucleotide sequence according to the invention;

b) if need be, recovery of said recombinant polypeptide.

When the procedure for preparation of a polypeptide of the invention employs a transgenic animal according to the invention, the recombinant polypeptide is then extracted from said animal.

The invention also relates to a polypeptide which is capable of being obtained by a procedure of the invention such as described previously.

The invention also comprises a procedure for preparation of a synthetic polypeptide, characterized in that it uses a sequence of amino acids of polypeptides according to the invention.

The invention likewise relates to a synthetic polypeptide obtained by a procedure according to the invention.

The polypeptides according to the invention can likewise be prepared by techniques which are conventional in the field of the synthesis of peptides. This synthesis can be carried out in homogeneous solution or in solid phase.

For example, recourse can be made to the technique of synthesis in homogeneous solution described by Houben-Weyl in 1974.

This method of synthesis consists in successively condensing, two by two, the successive amino acids in the order required, or in condensing amino acids and fragments formed previously and already containing several amino acids in the appropriate order, or alternatively several fragments previously prepared in this way, it being understood that it will be necessary to protect beforehand all the reactive functions carried by these amino acids or fragments, with the exception of amine functions of one and carboxyls of the other or vice-versa, which must normally be involved in the formation of peptide bonds, especially after activation

of the carboxyl function, according to the methods well known in the synthesis of peptides.

According to another preferred technique of the invention, recourse will be made to the technique described by Merrifield.

To make a peptide chain according to the Merrifield procedure, recourse is made to a very porous polymeric resin, on which is immobilized the first C-terminal amino acid of the chain. This amino acid is immobilized on a resin through its carboxyl group and its amine function is protected. The amino acids which are going to form the peptide chain are thus immobilized, one after the other, on the amino group, which is deprotected beforehand each time, of the portion of the peptide chain already formed, and which is attached to the resin. When the whole of the desired peptide chain has been formed, the protective groups of the different amino acids forming the peptide chain are eliminated and the peptide is detached from the resin with the aid of an acid.

The invention additionally relates to hybrid polypeptides having at least one polypeptide according to the invention, and a sequence of a polypeptide capable of inducing an immune response in man or animals.

Advantageously, the antigenic determinant is such that it is capable of inducing a humoral and/or cellular response.

It will be possible for such a determinant to comprise a polypeptide according to the invention in glycosylated form used with a view to obtaining immunogenic compositions capable of inducing the synthesis of antibodies directed against multiple epitopes. Said polypeptides or their glycosylated fragments are likewise part of the invention.

These hybrid molecules can be formed, in part, of a polypeptide carrier molecule or of fragments thereof according to the invention, associated with a possibly immunogenic part, in particular an epitope of the diphtheria toxin, the tetanus toxin, a surface antigen of the hepatitis B virus (patent FR 79 21811), the

VP1 antigen of the poliomyelitis virus or any other viral or bacterial toxin or antigen.

The procedures for synthesis of hybrid molecules encompass the methods used in genetic engineering for constructing hybrid nucleotide sequences coding for the polypeptide sequences sought. It will be possible, for example, to refer advantageously to the technique for obtainment of genes coding for fusion proteins described by Minton in 1984.

Said hybrid nucleotide sequences coding for a hybrid polypeptide as well as the hybrid polypeptides according to the invention characterized in that they are recombinant polypeptides obtained by the expression of said hybrid nucleotide sequences are likewise part of the invention.

The invention likewise comprises the vectors characterized in that they contain one of said hybrid nucleotide sequences. The host cells transformed by said vectors, the transgenic animals comprising one of said transformed cells as well as the procedures for preparation of recombinant polypeptides using said vectors, said transformed cells and/or said transgenic animals are, of course, likewise part of the invention.

The polypeptides according to the invention, the antibodies according to the invention described below and the nucleotide sequences according to the invention can advantageously be employed in procedures for the detection and/or identification of PWD circovirus, or of porcine circovirus other than a PWD circovirus, in a biological sample (biological tissue or fluid) capable of containing them. These procedures, according to the specificity of the polypeptides, the antibodies and the nucleotide sequences according to the invention which will be used, will in particular be able to detect and/or to identify a PWD circovirus or a porcine circovirus other than a PWD circovirus or other than the PWD circovirus of type B.

The polypeptides according to the invention can advantageously be employed in a procedure for the detection and/or the identification of PWD circovirus of type

A, of type B, of type A or B, or porcine circovirus other than the PWD circovirus of type B, or of porcine circovirus other than the PWD circovirus of type A or B, in a biological sample (biological tissue or fluid) capable of containing them, characterized in that it comprises the following steps:

a) contacting of this biological sample with a polypeptide or one of its fragments according to the invention (under conditions allowing an immunological reaction between said polypeptide and the antibodies possibly present in the biological sample);

b) demonstration of the antigen-antibody complexes possibly formed.

In the present description, PWD circovirus, except if a particular mention is indicated, will be understood as designating a PWD circovirus of type A or of type B, and porcine circovirus other than PWD, except if a particular mention is indicated, will be understood as designating a porcine circovirus other than a PWD circovirus of type A and B.

Preferably, the biological sample is formed by a fluid, for example a pig serum, whole blood or biopsies.

Any conventional procedure can be employed for carrying out such a detection of the antigen-antibody complexes possibly formed.

By way of example, a preferred method brings into play immunoenzymatic processes according to the ELISA technique, by immunofluorescence, or radioimmunological processes (RIA) or their equivalent.

Thus, the invention likewise relates to the polypeptides according to the invention, labeled with the aid of an adequate label such as of the enzymatic, fluorescent or radioactive type.

Such methods comprise, for example, the following steps:

- deposition of determined quantities of a polypeptide composition according to the invention in the wells of a microtiter plate,
- introduction into said wells of increasing dilutions of serum, or of a biological sample other than that defined previously, having to be analyzed,

- incubation of the microplate,
- introduction into the wells of the microtiter plate of labeled antibodies directed against pig immunoglobulins, the labeling of these antibodies having been carried out with the aid of an enzyme selected from those which are capable of hydrolyzing a substrate by modifying the absorption of the radiation of the latter, at least at a determined wavelength, for example at 550 nm,
- detection, by comparison with a control test, of the quantity of hydrolyzed substrate.

The invention likewise relates to a kit or set for the detection and/or identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, characterized in that it comprises the following elements:

- a polypeptide according to the invention,
- if need be, the reagents for the formation of the medium favorable to the immunological or specific reaction,
- if need be, the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction between the polypeptide(s) of the invention and the antibodies possibly present in the biological sample, these reagents likewise being able to carry a label, or to be recognized in their turn by a labeled reagent, more particularly in the case where the polypeptide according to the invention is not labeled,
- if need be, a biological reference sample (negative control) devoid of antibodies recognized by a polypeptide according to the invention,
- if need be, a biological reference sample (positive control) containing a predetermined quantity of antibodies recognized by a polypeptide according to the invention.

The polypeptides according to the invention allow monoclonal or polyclonal antibodies to be prepared which are characterized in that they specifically recognize

the polypeptides according to the invention. It will advantageously be possible to prepare the monoclonal antibodies from hybridomas according to the technique described by Kohler and Milstein in 1975. It will be possible to prepare the polyclonal antibodies, for example, by immunization of an animal, in particular a mouse, with a polypeptide or a DNA, according to the invention, associated with an adjuvant of the immune response, and then purification of the specific antibodies contained in the serum of the immunized animals on an affinity column on which the polypeptide which has served as an antigen has previously been immobilized. The polyclonal antibodies according to the invention can also be prepared by purification, on an affinity column on which a polypeptide according to the invention has previously been immobilized, of the antibodies contained in the serum of pigs infected by a PWD circovirus.

The invention likewise relates to mono- or polyclonal antibodies or their fragments, or chimeric antibodies, characterized in that they are capable of specifically recognizing a polypeptide according to the invention.

It will likewise be possible for the antibodies of the invention to be labeled in the same manner as described previously for the nucleic probes of the invention, such as a labeling of enzymatic, fluorescent or radioactive type.

The invention is additionally directed at a procedure for the detection and/or identification of PWD circovirus, of porcine circovirus other than a PWD circovirus, or other than the PWD circovirus of type B, in a biological sample, characterized in that it comprises the following steps:

a) contacting of the biological sample (biological tissue or fluid) with a mono- or polyclonal antibody according to the invention (under conditions allowing an immunological reaction between said antibodies and the polypeptides of PWD circovirus, of porcine circovirus other than a PWD circovirus, of porcine circovirus other than the PWD circovirus of type B, possibly present in the biological sample);

b) demonstration of the antigen-antibody complex possibly formed.

Likewise within the scope of the invention is a kit or set for the detection and/or the identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, characterized in that it comprises the following components:

- a polyclonal or monoclonal antibody according to the invention, if need be labeled;
- if need be, a reagent for the formation of the medium favorable to the carrying out of the immunological reaction;
- if need be, a reagent allowing the detection of the antigen-antibody complexes produced by the immunological reaction, this reagent likewise being able to carry a label, or being capable of being recognized in its turn by a labeled reagent, more particularly in the case where said monoclonal or polyclonal antibody is not labeled;
- if need be, reagents for carrying out the lysis of cells of the sample tested.

The present invention likewise relates to a procedure for the detection and/or the identification of PWD, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, in a biological sample, characterized in that it employs a nucleotide sequence according to the invention.

More particularly, the invention relates to a procedure for the detection and/or the identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, in a biological sample, characterized in that it contains the following steps:

a) if need be, isolation of the DNA from the biological sample to be analyzed;

b) specific amplification of the DNA of the sample with the aid of at least one primer, or a pair of primers, according to the invention;

c) demonstration of the amplification products.

These can be detected, for example, by the technique of molecular hybridization utilizing a nucleic probe according to the invention. This probe will

advantageously be labeled with a nonradioactive (cold probe) or radioactive element.

For the purposes of the present invention, "DNA of the biological sample" or "DNA contained in the biological sample" will be understood as meaning either the DNA present in the biological sample considered, or possibly the cDNA obtained after the action of an enzyme of reverse transcriptase type on the RNA present in said biological sample.

Another aim of the present invention consists in a procedure according to the invention, characterized in that it comprises the following steps:

a) contacting of a nucleotide probe according to the invention with a biological sample, the DNA contained in the biological sample having, if need be, previously been made accessible to hybridization under conditions allowing the hybridization of the probe with the DNA of the sample;

b) demonstration of the hybrid formed between the nucleotide probe and the DNA of the biological sample.

The present invention also relates to a procedure according to the invention, characterized in that it comprises the following steps:

a) contacting of a nucleotide probe immobilized on a support according to the invention with a biological sample, the DNA of the sample having, if need be, previously been made accessible to hybridization, under conditions allowing the hybridization of the probe with the DNA of the sample;

b) contacting of the hybrid formed between the nucleotide probe immobilized on a support and the DNA contained in the biological sample, if need be after elimination of the DNA of the biological sample which has not hybridized with the probe, with a nucleotide probe labeled according to the invention;

c) demonstration of the novel hybrid formed in step b).

According to an advantageous embodiment of the procedure for detection and/or identification defined previously, this is characterized in that, prior to step

a), the DNA of the biological sample is first amplified with the aid of at least one primer according to the invention.

The invention is additionally directed at a kit or set for the detection and/or the identification of PWD circovirus, of porcine circovirus other than the PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, characterized in that it comprises the following elements:

a) a nucleotide probe according to the invention;

b) if need be, the reagents necessary for the carrying out of a hybridization reaction;

c) if need be, at least one primer according to the invention as well as the reagents necessary for an amplification reaction of the DNA.

The invention likewise relates to a kit or set for the detection and/or the identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, characterized in that it comprises the following components:

a) a nucleotide probe, called a capture probe, according to the invention;

b) an oligonucleotide probe, called a revealing probe, according to the invention,

c) if need be, at least one primer according to the invention, as well as the reagents necessary for an amplification reaction of the DNA.

The invention also relates to a kit or set for the detection and/or identification of PWD circovirus, of porcine circovirus other than a PWD circovirus or of porcine circovirus other than the PWD circovirus of type B, characterized in that it comprises the following elements:

a) at least one primer according to the invention;

b) if need be, the reagents necessary for carrying out a DNA amplification reaction;

c) if need be, a component allowing the sequence of the amplified fragment to be verified, more particularly an oligonucleotide probe according to the invention.

The invention additionally relates to the use of a nucleotide sequence according to the invention, of a polypeptide according to the invention, of an antibody according to the invention, of a cell according to the invention, and/or of an animal transformed according to the invention, for the selection of an organic or inorganic compound capable of modulating, inducing or inhibiting the expression of genes, and/or of modifying the cellular replication of PWD circovirus or capable of inducing or of inhibiting the pathologies linked to an infection by a PWD circovirus.

The invention likewise comprises a method of selection of compounds capable of binding to a polypeptide or one of its fragments according to the invention, capable of binding to a nucleotide sequence according to the invention, or capable of recognizing an antibody according to the invention, and/or capable of modulating, inducing or inhibiting the expression of genes, and/or of modifying the cellular replication of PWD circovirus or capable of inducing or inhibiting the pathologies linked to an infection by a PWD circovirus, characterized in that it comprises the following steps:

a) contacting of said compound with said polypeptide, said nucleotide sequence, or with a cell transformed according to the invention and/or administration of said compound to an animal transformed according to the invention;

b) determination of the capacity of said compound to bind to said polypeptide or said nucleotide sequence, or to modulate, induce or inhibit the expression of genes, or to modulate the growth or the replication of PWD circovirus, or to induce or inhibit in said transformed animal the pathologies linked to an infection by PWD circovirus (designated activity of said compound).

The compounds capable of being selected can be organic compounds such as polypeptides or carbohydrates or any other organic or inorganic compounds already

known, or novel organic compounds elaborated by molecular modelling techniques and obtained by chemical or biochemical synthesis, these techniques being known to the person skilled in the art.

It will be possible to use said selected compounds to modulate the cellular replication of PWD circovirus and thus to control infection by this virus, the methods allowing said modulations to be determined being well known to the person skilled in the art.

This modulation can be carried out, for example, by an agent capable of binding to a protein and thus of inhibiting or of potentiating its biological activity, or capable of binding to an envelope protein of the external surface of said virus and of blocking the penetration of said virus into the host cell or of favoring the action of the immune system of the infected organism directed against said virus. This modulation can likewise be carried out by an agent capable of binding to a nucleotide sequence of a DNA of said virus and of blocking, for example, the expression of a polypeptide whose biological or structural activity is necessary for the replication or for the proliferation of said virus host cells to host cells in the host animal.

The invention relates to the compounds capable of being selected by a selection method according to the invention.

The invention likewise relates to a pharmaceutical composition comprising a compound selected from the following compounds:

a) a nucleotide sequence according to the invention;

b) a polypeptide according to the invention;

c) a vector, a viral particle or a cell transformed according to the invention;

d) an antibody according to the invention;

e) a compound capable of being selected by a selection method according to the invention;

possibly in combination with a pharmaceutically acceptable vehicle and, if need be, with one or more adjuvants of the appropriate immunity.

The invention also relates to an immunogenic and/or vaccine composition, characterized in that it comprises a compound selected from the following compounds:

a) a nucleotide sequence according to the invention;

b) a polypeptide according to the invention;

c) a vector or a viral particle according to the invention; and

d) a cell according to the invention.

In one embodiment, the vaccine composition according to the invention is characterized in that it comprises a mixture of at least two of said compounds a), b), c) and d) above and in that one of the two said compounds is related to the PWD circovirus of type A and the other is related to the PWD circovirus of type B.

In another enbodiment of the invention, the vaccine composition is characterized in that it comprises at least one compound a), b), c), or d) above which is related to PWD circovirus of type B. In still another embodiment, the the vaccine composition is characterized in that it comprises at least one compound a), b), c), or d) above which is related to PWD circovirus of type B ORF'2.

A compound related to the PWD circovirus of type A or of type B is understood here as respectively designating a compound obtained from the genomic sequence of the PWD circovirus of type A or of type B.

The invention is additionally aimed at an immunogenic and/or vaccine composition, characterized in that it comprises at least one of the following compounds:

- a nucleotide sequence SEQ ID No. 11, SEQ ID No. 12, or one of their fragments or homologues;
- a polypeptide of sequence SEQ ID No. 14, SEQ ID No. 15, or one of their fragments, or a modification thereof;

- a vector or a viral particle comprising a nucleotide sequence SEQ ID No. 11, SEQ ID No. 12, or one of their fragments or homologues;
- a transformed cell capable of expressing a polypeptide of sequence SEQ ID No. 14, SEQ ID No. 15, or one of their fragments, or a modification thereof; or
- a mixture of at least two of said compounds.

The invention also comprises an immunogenic and/or vaccine composition according to the invention, characterized in that it comprises said mixture of at least two of said compounds as a combination product for simultaneous, separate or protracted use for the prevention or the treatment of infection by a PWD circovirus, especially of type B.

In a preferred embodiment, the vaccine composition according to the invention comprises the mixture of the following compounds:

- a pcDNA3 plasmid containing a nucleic acid of sequence SEQ ID No. 11;
- a pcDNA3 plasmid containing a nucleic acid of sequence SEQ ID No. 12;
- a pcDNA3 plasmid containing a nucleic acid coding for the GM-CSF protein;
- a recombinant baculovirus containing a nucleic acid of sequence SEQ ID No. 11;
- a recombinant baculovirus containing a nucleic acid of sequence SEQ ID No. 12; and
- if need be, an adjuvant of the appropriate immunity, especially the adjuvant AIF™.

The invention is likewise directed at a pharmaceutical composition according to the invention, for the prevention or the treatment of an infection by a PWD circovirus.

The invention is also directed at a pharmaceutical composition according to the invention for the prevention or the treatment of an infection by the PWD circovirus of type B.

The invention likewise concerns the use of a composition according to the invention, for the preparation of a medicament intended for the prevention or the treatment of infection by a PWD circovirus, preferably by the PWD circovirus of type B.

Under another aspect, the invention relates to a vector, a viral particle or a cell according to the invention, for the treatment and/or the prevention of a disease by gene therapy.

Finally, the invention comprises the use of a vector, of a viral particle or of a cell according to the invention for the preparation of a medicament intended for the treatment and/or the prevention of a disease by gene therapy.

The polypeptides of the invention entering into the immunogenic or vaccine compositions according to the invention can be selected by techniques known to the person skilled in the art such as, for example, depending on the capacity of said polypeptides to stimulate the T cells, which is translated, for example, by their proliferation or the secretion of interleukins, and which leads to the production of antibodies directed against said polypeptides.

In pigs, as in mice, in which a weight dose of the vaccine composition comparable to the dose used in man is administered, the antibody reaction is tested by taking of the serum followed by a study of the formation of a complex between the antibodies present in the serum and the antigen of the vaccine composition, according to the usual techniques.

The pharmaceutical compositions according to the invention will contain an effective quantity of the compounds of the invention, that is to say in sufficient quantity of said compound(s) allowing the desired effect to be obtained, such as, for example, the modulation of the cellular replication of PWD circovirus. The person skilled in the art will know how to determine this quantity, as a function, for example, of the age and of the weight of the individual to be treated, of the state of advancement of the pathology, of the possible secondary effects and by means of a

test of evaluation of the effects obtained on a population range, these tests being known in these fields of application.

According to the invention, said vaccine combinations will preferably be combined with a pharmaceutically acceptable vehicle and, if need be, with one or more adjuvants of the appropriate immunity.

Today, various types of vaccines are available for protecting animals or man against infectious diseases: attenuated living microorganisms (*M. bovis* - BCG for tuberculosis), inactivated microorganisms (influenza virus), acellular extracts (*Bordetella pertussis* for whooping cough), recombined proteins (surface antigen of the hepatitis B virus), polysaccharides (pneumococcal). Vaccines prepared from synthetic peptides or genetically modified microorganisms expressing heterologous antigens are in the course of experimentation. More recently still, recombined plasmid DNAs carrying genes coding for protective antigens have been proposed as an alternative vaccine strategy. This type of vaccination is carried out with a particular plasmid originating from a plasmid of *E.coli* which does not replicate *in vivo* and which codes uniquely for the vaccinating protein. Animals have been immunized by simply injecting the naked plasmid DNA into the muscle. This technique leads to the expression of the vaccine protein *in situ* and to an immune response of cellular type (CTL) and of humoral type (antibody). This double induction of the immune response is one of the principal advantages of the vaccination technique with naked DNA.

The vaccine compositions comprising nucleotide sequences or vectors into which are inserted said sequences are especially described in the international application No. WO 90/11092 and likewise in the international application No. WO 95/11307.

The constitutive nucleotide sequence of the vaccine composition according to the invention can be injected into the host after having been coupled to compounds which favor the penetration of this polynucleotide into the interior of the cell or its transport to the cell nucleus. The resultant conjugates can be encapsulated in

polymeric microparticles, as described in the international application No. WO 94/27238 (Medisorb Technologies International).

According to another embodiment of the vaccine composition according to the invention, the nucleotide sequence, preferably a DNA, is complexed with DEAE-dextran (Pagano et al., 1967) or with nuclear proteins (Kaneda et al., 1989), with lipids (Felgner et al., 1987) or encapsulated in liposomes (Fraley et al., 1980) or else introduced in the form of a gel facilitating its transfection into the cells (Midoux et al., 1993, Pastore et al., 1994). The polynucleotide or the vector according to the invention can also be in suspension in a buffer solution or be combined with liposomes.

Advantageously, such a vaccine will be prepared according to the technique described by Tacson et al. or Huygen et al. in 1996 or alternatively according to the technique described by Davis et al. in the international application No. WO 95/11307.

Such a vaccine can likewise be prepared in the form of a composition containing a vector according to the invention, placed under the control of regulation elements allowing its expression in man or animal. It will be possible, for example, to use, by way of *in vivo* expression vector of the polypeptide antigen of interest, the plasmid pcDNA3 or the plasmid pcDNA1/neo, both marketed by Invitrogen (R&D Systems, Abingdon, United Kingdom). It is also possible to use the plasmid V1Jns.tPA, described by Shiver et al. in 1995. Such a vaccine will advantageously comprise, apart from the recombinant vector, a saline solution, for example a sodium chloride solution.

Pharmaceutically acceptable vehicle is understood as designating a compound or a combination of compounds entering into a pharmaceutical composition or vaccine which does not provoke secondary reactions and which allows, for example, the facilitation of the administration of the active compound, an increase in its duration of life and/or its efficacy in the body, an increase in its solubility in solution or alternatively an improvement in its conservation. These pharmaceutically

acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the nature and of the mode of administration of the chosen active compound.

As far as the vaccine formulations are concerned, these can comprise adjuvants of the appropriate immunity which are known to the person skilled in the art, such as, for example, aluminum hydroxide, a representative of the family of muramyl peptides such as one of the peptide derivatives of N-acetyl muramyl, a bacterial lysate, or alternatively Freund's incomplete adjuvant.

These compounds can be administered by the systemic route, in particular by the intravenous route, by the intramuscular, intradermal or subcutaneous route, or by the oral route. In a more preferred manner, the vaccine composition comprising polypeptides according to the invention will be administered by the intramuscular route, through the food or by nebulization several times, staggered over time.

Their administration modes, dosages and optimum pharmaceutical forms can be determined according to the criteria generally taken into account in the establishment of a treatment adapted to an animal such as, for example, the age or the weight, the seriousness of its general condition, the tolerance to the treatment and the secondary effects noted. Preferably, the vaccine of the present invention is administered in an amount that is protective against piglet weight loss disease. See, e.g., "Immunization Practice Advisory Committee," *Clinical Pharmacy* 8: 839-851 (1989).

For example, in the case of a vaccine according to the present invention comprising a nucleotide sequence of the genome of PCV, or a homologue or fragment thereof, the nucleotide sequence will be administered one time or several times, spread out over time, in an amount between about 5 to about 320 μg per kilogram of the weight of the animal, preferably about 10 to about 160 μg/kg, and more preferably about 20 to about 80 μg/kg for a dose.

For example, in the case of a vaccine according to the present invention comprising a polypeptide encoded by a nucleotide sequence of the genome of PCV,

or a homolgue or fragment thereof, the polypeptide will be administered one time or several times, spread out over time, directly or by means of a transformed cell capable of expressing the polypeptide, in an amount of about 0.1 to 10 μg per kilogram weight of the animal, prefereably about 0.2 to about 5 μg/kg, more preferably about 0.5 to about 2 μg/kg for a dose.

The present invention likewise relates to the use of nucleotide sequences of PWD circovirus according to the invention for the construction of autoreplicative retroviral vectors and the therapeutic applications of these, especially in the field of human gene therapy in vivo.

The feasibility of gene therapy applied to man no longer needs to be demonstrated and this relates to numerous therapeutic applications like genetic diseases, infectious diseases and cancers. Numerous documents of the prior art describe the means of employing gene therapy, especially through viral vectors. Generally speaking, the vectors are obtained by deletion of at least some of the viral genes which are replaced by the genes of therapeutic interest. Such vectors can be propagated in a complementation line which supplies in trans the deleted viral functions in order to generate a defective viral vector particle for replication but capable of infecting a host cell. To date, the retroviral vectors are amongst the most widely used and their mode of infection is widely described in the literature accessible to the person skilled in the art.

The principle of gene therapy is to deliver a functional gene, called a gene of interest, of which the RNA or the corresponding protein will produce the desired biochemical effect in the targeted cells or tissues. On the one hand, the insertion of genes allows the prolonged expression of complex and unstable molecules such as RNAs or proteins which can be extremely difficult or even impossible to obtain or to administer directly. On the other hand, the controlled insertion of the desired gene into the interior of targeted specific cells allows the expression product to be regulated in defined tissues. For this, it is necessary to be able to insert the desired

therapeutic gene into the interior of chosen cells and thus to have available a method of insertion capable of specifically targeting the cells or the tissues chosen.

Among the methods of insertion of genes, such as, for example, microinjection, especially the injection of naked plasmid DNA (Derse, D. et al., 1995, and Zhao, T.M. et al., 1996), electroporation, homologous recombination, the use of viral particles, such as retroviruses, is widespread. However, applied in vivo, the gene transfer systems of recombinant retroviral type at the same time have a weak infectious power (insufficient concentration of viral particles) and a lack of specificity with regard to chosen target cells.

The production of cell-specific viral vectors, having a tissue-specific tropism, and whose gene of interest can be translated adequately by the target cells, is realizable, for example, by fusing a specific ligand of the target host cells to the N-terminal part of a surface protein of the envelope of PWD circovirus. It is possible to mention, for example, the construction of retroviral particles having the CD4 molecule on the surface of the envelope so as to target the human cells infected by the HIV virus (YOUNG, J.A.T. et al., Sciences 1990, 250, 1421-1423), viral particles having a peptide hormone fused to an envelope protein to specifically infect the cells expressing the corresponding receptor (KASAHARA, N. et al., Sciences 1994, 266, 1373-1376) or else alternatively viral particles having a fused polypeptide capable of immobilizing on the receptor of the epidermal growth factor (EGF) (COSSET, F.L. et al., J. of Virology 1995, 69, 10, 6314-6322). In another approach, single-chain fragments of antibodies directed against surface antigens of the target cells are inserted by fusion with the N-terminal part of the envelope protein (VALSESIA-WITTMAN, S. et al., J. of Virology 1996, 70, 3, 2059-2064; TEARINA CHU, T.H. et al., J. of Virology 1997, 71, 1, 720-725).

For the purposes of the present invention, a gene of interest in use in the invention can be obtained from a eukaryotic or prokaryotic organism or from a virus by any conventional technique. It is, preferably, capable of producing an expression product having a therapeutic effect and it can be a product homologous to the cell

host or, alternatively, heterologous. In the scope of the present invention, a gene of interest can code for an (i) intracellular or (ii) membrane product present on the surface of the host cell or (iii) secreted outside the host cell. It can therefore comprise appropriate additional elements such as, for example, a sequence coding for a secretion signal. These signals are known to the person skilled in the art.

In accordance with the aims pursued by the present invention, a gene of interest can code for a protein corresponding to all or part of a native protein as found in nature. It can likewise be a chimeric protein, for example arising from the fusion of polypeptides of various origins or from a mutant having improved and/or modified biological properties. Such a mutant can be obtained, by conventional biological techniques, by substitution, deletion and/or addition of one or more amino acid residues.

It is very particularly preferred to employ a gene of therapeutic interest coding for an expression product capable of inhibiting or retarding the establishment and/or the development of a genetic or acquired disease. A vector according to the invention is in particular intended for the prevention or for the treatment of cystic fibrosis, of hemophilia A or B, of Duchenne's or Becker's myopathy, of cancer, of AIDS and of other bacteria or infectious diseases due to a pathogenic organism: virus, bacteria, parasite or prion. The genes of interest utilizable in the present invention are those which code, for example, for the following proteins:

- a cytokine and especially an interleukin, an interferon, a tissue necrosis factor and a growth factor and especially a hematopoietic growth factor (G-CSF, GM-CSF),
- a factor or cofactor involved in clotting and especially factor VIII, von Willebrand's factor, antithrombin III, protein C, thrombin and hirudin,
- an enzyme or an enzyme inhibitor such as the inhibitors of viral proteases,
- an expression product of a suicide gene such as thymidine kinase of the HSV virus (herpesvirus) of type 1,
- an activator or an inhibitor of ion channels,

- a protein of which the absence, the modification or the deregulation of expression is responsible for a genetic disease, such as the CFTR protein, dystrophin or minidystrophin, insulin, ADA (adenosine diaminose), glucocerebrosidase and phenylhydroxylase,
- a protein capable of inhibiting the initiation or the progression of cancers, such as the expression products of tumor suppressor genes, for example the P53 and Rb genes,
- a protein capable of stimulating an immune or an antibody response, and
- a protein capable of inhibiting a viral infection or its development, for example the antigenic epitopes of the virus in question or altered variants of viral proteins capable of entering into competition with the native viral proteins.

The invention thus relates to the vectors characterized in that they comprise a nucleotide sequence of PWD circovirus according to the invention, and in that they additionally comprise a gene of interest.

The present invention likewise relates to viral particles generated from said vector according to the invention. It additionally relates to methods for the preparation of viral particles according to the invention, characterized in that they employ a vector according to the invention, including viral pseudoparticles (VLP, virus-like particles).

The invention likewise relates to animal cells transfected by a vector according to the invention.

Likewise comprised in the invention are animal cells, especially mammalian, infected by a viral particle according to the invention.

The present invention likewise relates to a vector, a viral particle or a cell according to the invention, for the treatment and/or the prevention of a genetic disease or of an acquired disease such as cancer or an infectious disease. The invention is likewise directed at a pharmaceutical composition comprising, by way

of therapeutic or prophylactic agent, a vector or a cell according to the invention, in combination with a vehicle acceptable from a pharmaceutical point of view.

Other characteristics and advantages of the invention appear in the examples and the figures.

The invention is described in more detail in the following illustrative examples. Although the examples may represent only selected embodiments of the invention, it should be understood that the following examples are illustrative and not limiting.

Examples

EXAMPLE 1: Cloning, sequencing and characterization of the PWD circovirus of type A (PCVA)

1. Experimental procedures

Experimental reproduction of the infection and its syndrome (cf. Figure 1).

A first test was carried out with pigs from a very well-kept farm, but affected by piglet weight loss disease (PWD), likewise called fatal piglet wasting (FPW). Tests carried out with SPF (specific pathogen-free) pigs showed a transfer of contaminant(s) finding expression in a complex pathology combining hyperthermia, retardation of growth, diarrhea and conjunctivitis. The PDRS (porcine dysgenic and respiratory syndrome) virus, an infectious disease due to an arteriovirus) was rapidly isolated from breeding pigs and contact pigs. It should have been possible to attribute all the clinical signs to the presence of the PDRS virus. However, two farm pigs presented signs of FPW without the PDRS virus being isolated. The histological analyses and blood formulas, however, showed that these pigs were suffering from an infectious process of viral origin.

In a second test, 8-week SPF pigs were inoculated by the intratracheal route with organ homogenates of two farm pigs suffering from FPW. The inoculated pigs

exhibited hyperthermia 8 to 9 days post-infection, then their growth was retarded. Other SPF pigs, placed in contact, had similar, attenuated signs 30 days after the initial experiment. No seroconversion with respect to a European or Canadian strain of PDRS virus was recorded in these animals.

A third test allowed the syndrome to be reproduced from samples taken from the pigs of the second test.

Conclusion

The syndrome is reproduced under the experimental conditions. It is determined by at least one infectious agent, which is transmittable by direct contact. The clinical constants are a sometimes high hyperthermia (greater than or equal to 41.5°C) which develops 8 to 10 days after infection. Retardation of the growth can be observed. The other signs are a reversal of the blood formula (reversal of the lymphocyte/polynuclear ratio from 70/30 to 30/70) and frequent lesions on the ganglia, especially those draining the respiratory apparatus (ganglionic hypertrophy, loss of structure with necrosis and infiltration by mononucleated or plurinucleated giant cells).

2. Laboratory studies

Various cell supports including primary pig kidney cells or cell lines, pig testicle cells, monkey kidney cells, pig lymphocytes, pig alveolar macrophages and circulating blood monocytes were used to demonstrate the possible presence of a virus. No cytopathic effect was demonstrated in these cells. On the other hand, the use of a serum of a pig sick after experimental infection allowed an intracellular antigen to be revealed in the monocytes, the macrophages and approximately 10% of pig kidney (PK) cells infected with organ homogenates. This indirect revealing was carried out kinetically at different culture times. It is evident from this that the antigen initially appears in the nucleus of the infected cells before spreading into the cytoplasm. The successive passages in cell culture did not allow the signal to be amplified.

Under electron microscopy on organ homogenates, spherical particles labeled specifically by the serum of sick pigs, infected under the experimental conditions, were visualized. The size of these particles is estimated at 20 nm.

After two passages of these organ homogenates over pig lymphocytes and then three passages over pig kidney or testicle cells, a cytopathic effect developed and was amplified. An adenovirus was visualized in the electron microscope, which, under the experimental conditions, did not reproduce FPW (only a hyperthermia peak was noted 24 to 48 hours after infection, and then nothing more).

It has been possible to demonstrate DNA bands in certain samples of pigs infected under the experimental conditions and having exhibited signs of the disease (results not shown). A certain connection exists between the samples giving a positive result in cell culture and those having a DNA band.

Conclusion

At least two types of virus were demonstrated in the organ homogenates from pigs suffering from FPW. One is an adenovirus, but by itself alone it does not reproduce the disease. The other type of virus is a circovirus and is associated with FPW. This circovirus, of which two types have been isolated and sequenced, designated below PWD circovirus type A (or PCVA) and PWD circovirus of type B (or PCVB) have mutations with respect to the known sequences of circovirus which are nonpathogenic for the pig.

3. Cloning and sequencing of the DNA of the PWD circovirus of type A

Extraction of the replicative form (RF) DNA, cleavage by the Kpn I enzyme and amplification by a pair of primers flanking the Kpn I restriction site. Sequencing of the two strands at least twice by the Sanger method.

The nucleic sequence of the strand of (+) polarity of the genome of the PWD circovirus of type A (or PCVA), strain FPW, is represented by the sequence SEQ ID No. 1 in the list of sequences, the nucleic sequence of the strand of (-) polarity of the genome of the PWD circovirus of type A (or PCVA) being

represented by the nucleic sequence 3' → 5' of Figure 3 or by the sequence SEQ ID No. 2 (represented according to the orientation 5' → 3') in the list of sequences.

The amino acid sequences SEQ ID No. 6, SEQ ID No. 7 and SEQ ID No. 8 of the list of sequences respectively represent the sequences of proteins encoded by the nucleic sequences of the 3 open reading frames SEQ ID No. 3 (ORF1), corresponding to the REP protein, SEQ ID No. 4 (ORF2) and SEQ ID No. 5 (ORF3), determined from the sequence SEQ ID No. 1 of the strand of (+) polarity or of the nucleic sequence SEQ ID No. 2 of the strand of (-) polarity of the genome of the PWD circovirus of type A.

4. Comparison of the nucleotide sequences and amino acids of the PWD circovirus of type A (or associated with PWD) which are obtained with the corresponding sequences of MEEHAN and MANKERTZ circoviruses of porcine cell lines

Use of the DNA sequence analysis software, DNASIS.

Sequences of oligonucleotides used as primers or probes in the detection and/or identification procedures

1. Specific detection of the PWD circovirus of type A:

primer PCV 5: 5' GTG TGC TCG ACA TTG GTG TG 3';

primer PCV 10: 5' TGG AAT GTT AAC GAG CTG AG 3';

2. Specific detection of the circovirus of the cell lines:

primer PCF 5: 5' GTG TGC TCG ACA TTG GTG TG 3';

primer MEE 1: 5' TGG AAT GTT AAC TAC CTC AA 3';

3. Differential detection:

the pairs of primers used are those described, for example, in the paragraphs 1 and 2 above;

4. Detection of the monomeric circular replicative forms RF:

primer PCV 5: 5' GTG TGC TCG ACA TTG GTG TG 3';

primer PCV 6: 5' CTC GCA GCC ATC TTG GAA TG 3';

5. Detection of the vectors carrying the dimers in tandem:

Nar dimer:

primer KS 620: 5' CGC GCG TAA TAC GAC TCA CT 3';

primer PCV 5: 5' GTG TGC TCG ACA TTG GTG TG 3';

Kpn dimer:

primer KS 620: 5' CGC GCG TAA TAC GAC TCA CT 3';

primer PCV 6: 5'CTC GCA GCC ATC TTG GAA TG 3';

6. Differential detection:

The pairs of primers used are those described, for example, in paragraphs 4 and 5 above.

The procedures using the pairs or primers described in paragraphs 4 and 5 are of particular interest for differentially detecting the circular monomeric forms of specific replicative forms of the virion or of the DNA in replication and the dimeric forms found in the so-called in-tandem molecular constructs.

The in-tandem constructs of the viral genome (dimers) such as the constructs used for the preparation of the pBS KS + tandem PCV Kpn I vector, deposited at the CNCM under the number I-1891, 3 July 1997 (E. coli transformed by said vector) are very interesting for their use in methods of production in sufficient quantity of an inoculum formed of DNA, intended for the virus production, this in the absence of a satisfactory virus production protocol in a cell system. These said methods of production using these in-tandem constructs of the viral genome will allow the virulence factors to be studied by mutation and by way of consequence will be able to be used for the production of a collection of viruses carrying the mutations indicated in the construction of vectors which will have the appropriate tropism and virulence. These vectors with autoreplicative structure have the sought gene transfer properties, especially for their applications in gene therapy, and in vaccinology.

Western-blot analysis of recombinant proteins of the PWD circovirus of type A

The results were obtained using a specific antiserum of the PWD circovirus produced during test 1 (cf. Figure 1).

Type of products analyzed.

The analyses were carried out on cell extracts of Sf9 cells obtained after infection by the recombinant baculovirus PCV ORF 1.

The culture of Sf9 cells was carried out in a 25 cm^2 Petri dish according to the standard culture methods for these cells. After centrifugation, the cell pellets are taken up with 300 μl of PBS buffer (phosphate saline buffer).

Electrophoresis (PAGE-SDS)

The electrophoresis is carried out on the cell extracts of Sf9 cells obtained previously on 5 samples (cf. Table 1 below) under the following conditions:

% polyacrylamide gel: 8%; conditions: denaturing

Voltage: 80 V; duration: 135 mn.

Table 1: Nature of the samples subjected to electrophoresis

Well No.	1	2	3	4	5
Sample applied	PM Rainbow	Raoul 24 h	Raoul 48 h	Raoul 72 h	Raoul 96 h
μl of sample	10	15	15	15	15
μl of Laemmli 4X	0	5	5	5	5

Legends to Table 1:

Laemmli 4X: loading buffer

PM Rainbow: molecular-weight markers (35, 52, 77, 107, 160 and 250 kD)

Raoul 24 h, 48 h, 72 h and 96 h: expression products of the ORF1 of the PWD circovirus of type A.

Western blot

After electrophoresis, the bands obtained in the different wells are transferred to nitrocellulose membrane for 1 h at 100 v in a TGM buffer (tris-glycine-methanol).

The Western blot is carried out under the following conditions:

1) Saturation with a solution containing 5% of skimmed milk; 0.05% of Tween 20 in a TBS 1X buffer (tris buffer saline) for 30 min.

2) 1st antibody:

10 ml of PWD anticircovirus antibody of type A are added diluted to 1/100, then the reaction mixture is incubated for one night at 4°C. Three washes of 10 min in TBS 1X are carried out.

3) 2nd antibody:

10 ml of pig rabbit P164 antibody anti-immunoglobulins, coupled to peroxidase (Dakopath) are added diluted to 1/100, then the reaction medium is incubated for 3 hours at 37°C. Three washes of 10 min in TBS 1X are carried out.

4) Visualization

The substrate 4-chloro-1-naphthol in the presence of oxygenated water is used for visualization.

Results

The results are shown in Figure 7.

Kinetics of appearance of antibodies specific for the REP recombinant protein of the PWD circovirus of type A expressed in baculovirus after infection of pigs by the PWD circovirus of type A (test 4, cf. Figure 1)

After infection of the pigs, a sample of serum of each of the infected pigs is taken at different periods expressed in the table by the date of taking (carried out here in the same year) and is then analyzed by Western blot.

The visualization of the specific antibodies is carried out in the manner described previously.

The results obtained are shown by Table 2 below.

Table 2: Kinetics of appearance of specific antibodies

Sample	Pigs	10/6	16/06	23/06	01/07	08/07	15/07	21/07
A3	1						Neg.	
Control	2						Neg.	
B2 Infec.	1	Neg.	Neg.	Neg.	+	+	++	+++
RP+	2	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	3	Neg.	Neg.	Neg.	Neg.	+	+	+
	4	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	++

Legends to Table 2:

A3 control: uninfected control animals;
B2 Infec. RP+: animals infected with pig kidney (PK) cells containing the circovirus;
Neg.: negative;
+, ++, +++: intensity scale of the positive reaction;
10/06, 16/06, 23/06, 01/07, 08/07, 15/07, 21/07: dates expressed in day/month on which the different withdrawals of serum were carried out.

EXAMPLE 2: Cloning, sequencing and characterization of the type B PWD circovirus (PCVB)

The techniques used for cloning, sequencing and characterization of the type B PWD circovirus (PCVB) are those used in Example 1 above for the type A PWD circovirus (PCVA).

The nucleic sequence of the strand of (+) polarity of the genome of the PWD circovirus of type B (or PCVB) is represented by the sequence SEQ ID No. 9 in the sequence listing, the nucleic sequence of the strand of (-) polarity of the genome of the PWD circovirus of type B (or PCVB) being represented by the nucleic sequence 3' → 5' of Figure 8 or by the sequence SEQ ID No. 10 (represented according to the orientation 5' → 3') in the sequence listing.

The amino acid sequences SEQ ID No. 14, SEQ ID No. 15 and SEQ ID No. 16 of the sequence listing respectively represent the sequences of the proteins encoded by the nucleic sequences of the 3 open reading frames SEQ ID No. 11 (ORF'1), corresponding to the REP protein, SEQ ID No. 12 (ORF'2) and SEQ ID No. 13 (ORF'3), determined from the sequence SEQ ID No. 9 of the strand of (+) polarity or from the nucleic sequence SEQ ID No. 10 of the strand of (-) polarity of the genome of the PWD circovirus of type B.

EXAMPLE 3: Comparative analysis of nucleotide sequences (ORF1, ORF2 and genomic) and amino acid sequences encoded by the ORF1 and the ORF2 of the PWD circoviruses of type A (PCVA) and of type B (PCVB)

The results expressed in % of homology are shown in Tables 3 and 4 below.

Table 3: Compared analysis of the amino acid sequences

% homology	ORF1	ORF2
PCVA/PCVB	80.4	56.2

Table 4: Compared analysis of the nucleotide sequences

% homology	Genomic	ORF1	ORF2	The remainder
PCVA/PCVB	70.4	80.4	60.1	66.1

EXAMPLE 4: Observation of the disease and reproduction of the disease under experimental conditions

a) Test No. 1: Observation of the disease

The objective is to take breeding animals at the start of disease and to place them under experimental conditions to follow the progression of the pathology and describe all the clinical signs thereof. This first test was carried out on 3 breeding pigs aged 10 weeks of which 2 were already ill (suffering from wasting), and on 3 other pigs aged 13 weeks, not having signs of disease. The clinical observation was spread over a period of 37 days. Two pigs of 10 weeks wasted rapidly (pigs 1 and 2, Figure 9) and had to be painlessly killed 5 and 6 days after their arrival. A single pig exhibited hyperthermia over 5 days and diarrhea. Two other pigs exhibited dyspnea and cough, of which one additionally had hyperthermia, greater than 41°C, for the two first days of its stay. Another pig had retarded growth in the second week (pig 6, Figure 9), without any other clinical sign being recorded. On the lesional level, 5 pigs out of 6 exhibited macroscopic lesions of gray pneumonia, the sixth exhibited cicatricial lesions on the lung.

b) Test No. 2: Reproduction of the disease from inocula prepared in farm pigs.

The two sick pigs in test 1 served to prepare inocula which were tested in test 2 on specific-pathogen-free (SPF) pigs. The SPF pigs were aged 9 weeks at the time of inoculation. The clinical and lesional results are shown in Table 5.

Table 5: Summary of the measurements carried out during experimental reproduction of PWD. (The values of the control animals are reported in brackets, the underlined values indicate a difference between infected animals and control animals)

Test / Measurement	2	3	4	5	6	7
Status of the pigs	SPF CNEVA	SPF field	SPF CNEVA	SPF CNEVA	Conventional	Conventional
Age	9 weeks	6 weeks	5 weeks	5 weeks	5 weeks	6-7 weeks
Number	4	6	12	8	8	8
Inoculation route	Intratracheal route	Intratracheal route	Intratracheal + intramuscular route	Intratracheal + intramuscular route	Intratracheal + intramuscular route	Intratracheal + intramuscular route
Inoculum titer per pig	ND*	ND*	$10^{4\,53}$ $TCID_{50}$ per ml: 1 ml IM + 5 ml IT	$10^{4.53}$ $TCID_{50}$ per ml: 1 ml IM + 5 ml IT	$10^{4\,53}$ $TCID_{50}$ per ml: 1 ml IM + 5 ml IT	$10^{4.53}$ $TCID_{50}$ per ml: 1 ml IM + 5 ml IT
Start of hyperthermia	10 days post-infection	9-13 days post-infection	12-13 days post-infection	9-14 days post-infection	8-12 days post-infection	12 days post-infection
% of pigs in hyperthermia**	100%	83%	92%	100%	75%	88%
Number of days of hyperthermia per pig**	7	4.5	3.3	5.8	7.5	11.6
Maximum temperatures	40.4 to 41.7°C	40.6 to 42.3°C	40.2 to 41.6°C	40.3 to 40.8°C	40.6 to 42°C	40.2 to 41.9°C

Test	2	3	4	5	6	7
Measurement ***						
Hyperthermia****						
% per week						
W1	3.5 (3.5)	17 (36)	7 (5)	37 (17)	16 (17)	20 (28)
W2	42 (3.5)	7 (13)	13 (1)	21 (3)	52 (10)	37 (28)
W3	35 (3.5)	33 (10)	28 (7)	62 (2)	34 (12)	79 (17)
W4	21 (3.5)	28 (7)	5 (0)	6 (3)	25 (22)	55 (3)
DMG:						
W1	928 (1053)	417 (357)	564 (620)	650 (589)	401 (407)	509 (512)
W2	678 (1028)	428 (617)	503 (718)	612 (584)	294 (514)	410 (310)
W3	661 (1000)	771 (642)	381 (657)	520 (851)	375 (586)	435 (440)
W4	786 (1100)	550 (657)	764 (778)	641 (696)	473 (610)	451 (681)
Contact pigs	Yes to 100%	Yes to 75%	Not tested	Not tested	Not tested	Not tested
transmission						
% of pulmonary lesions	25	75	0	25	25	12
% of ganglionic lesions	17	33	67	25	50	12

* ND: not determined,

** hyperthermia when the temperature is greater than 40°C,

*** range of maximum temperatures recorded at the individual level,

**** the percentage corresponds to the number of temperature recordings greater than 40°C divided by the total number of temperature recordings in the week on all of the pigs.

In this test, there was no wasting, at the very most a retardation of the growth in the second, third or fourth week after infection. These data illustrate that certain breeding conditions probably favor the expression of the disease.

c) Tests No. 3 to No. 7: Reproduction of the experimental tests

The increase in the number of the experimental tests on pigs had the mastering and better characterization of the experimental model as an objective. All of the results are presented in Table 5.

Under the experimental conditions, PWD is thus characterized by a long incubation, of 8 to 14 days, true hyperthermia over 2 to 8 days, a decrease in food consumption and a retardation of the increase in weight on the second, third or fourth week post-infection. The lesional table associated with this clinical expression includes, in the main, ganglionic hypertrophy and lesions of pneumonia.

Conclusion

The perfection of this experimental model allows the direct etiological role of the PWD circovirus in the disease to be indisputably demonstrated. In addition, this model is an indispensable tool for the understanding of pathogenic mechanisms and the study of future vaccine candidates.

EXAMPLE 5: Demonstration of the vaccine composition protective efficacy produced from nucleic fragments of PWD circovirus sequence

1) Animals used for the study

Piglets having the PWD disease, reproduced under experimental conditions described in paragraph c) of Example 4, were used in a protocol for evaluating the vaccine composition efficacy, comprising nucleic fragments of PWD circovirus sequence.

2) Tested vaccine composition and vaccination protocol

a) Components used for the study

The plasmids were obtained from the pcDNA3 plasmid of INVITROGENE - pcDNA3ORF- plasmids

These plasmids are plasmids which do not carry a PWD circovirus nucleic acid insert and are used as a negative control plasmid.

- pcDNA3ORF1+ plasmid and pcDNA3ORF2+ plasmid

The pcDNA3ORF1+ and pcDNA3ORF2+ plasmids are plasmids which carry a nucleic acid insert of the sequence of the PWD circovirus of TYPE B, respectively an insert comprising the nucleic acid fragment SEQ ID No. 11 (ORF'1) coding for the Rep protein of sequence SEQ ID No. 14 and an insert comprising the nucleic acid fragment SEQ ID No. 12 (ORF'2) coding for the protein of sequence SEQ ID No. 15, probably corresponding to the capsid protein, these nucleic constructs comprising the ATG initiation codon of the coding sequence of the corresponding protein.

- GMCSF+ plasmid

GM-CSF (granulocyte/macrophage colony stimulating factor) is a cytokine which occurs in the development, the maturation and the activation of macrophages, granulocytes and dendritic cells which present an antigen. The beneficial contribution of the GM-CSF in vaccination is considered to be a cellular activation with, especially, the recruitment and the differentiation of cells which present an antigen.

This pcDNA3-GMCSF+ plasmid carries a nucleic acid insert coding for the granulocyte/macrophage colony stimulation factor, the GM-CSF protein.

The gene coding for this GM-CSF protein was cloned and sequenced by Inumaru et al. (Immunol. Cell Biol., 1995, 73 (5), 474-476). The pcDNA3-GMCSF+ plasmid was obtained by Dr. B. Charley of INRA of Jouy-en-Josas (78, France).

- Recombinant baculoviruses

The so-called ORF- baculoviruses are viruses not carrying any insert comprising a nucleic acid fragment capable of expressing a PWD circovirus protein.

The so-called ORF1+ (BAC ORF1+) or ORF2+ (BAC ORF2+) baculoviruses are recombinant baculoviruses respectively carrying an insert

comprising a nucleic acid fragment SEQ ID No. 11 (ORF'1) and an insert comprising the nucleic acid fragment SEQ ID No. 12 (ORF'2).

- Adjuvant

The adjuvant supplied by the Seppic Company, a subsidiary of AIR LIQUIDE, is the adjuvant corresponding to the reference AIF SEPPIC.

b) Vaccination protocol

Weaned piglets aged 3 weeks are divided into four batches A, B, C and D each comprising 8 piglets.

Batches A, B and C, aged 3 weeks, each receive a first injection (injection M1) of 1 ml containing 200 micrograms of plasmids (naked DNA) in PBS, pH: 7.2, by the intramuscular route for each of the plasmids mentioned below for each batch, then, at the age of 5 weeks, a second injection (injection M2) comprising these same plasmids. A third injection is carried out simultaneously on the other side of the neck. This third injection comprises 1 ml of a suspension containing 5.10^6 cells infected by recombinant baculoviruses and 1 ml of AIF SEPPIC adjuvant.

Batch A (F1) (control batch):

- first injection

pcDNA3ORF1- plasmid, pcDNA3ORF2- plasmid and GMCSF+ plasmid.

- second and third injection (simultaneous)

pcDNA3ORF1- plasmid, pcDNA3ORF2- plasmid and GMCSF+ plasmid;

Cells transformed by baculoviruses not containing any nucleic acid insert coding for a PWD circovirus protein;

AIF SEPPIC adjuvant.

Batch B (F2) (control batch):

- first injection

pcDNA3ORF1- plasmid, pcDNA3ORF2- plasmid and GMCSF+ plasmid;

- second and third injection (simultaneous)

pcDNA3ORF1- plasmid, pcDNA3ORF2- plasmid and GMCSF+ plasmid;

Cells transformed by baculoviruses not containing any nucleic acid insert coding for a PWD circovirus protein;

AIF SEPPIC adjuvant.

Batch C (F3):

- first injection

pcDNA3ORF1+ plasmid, pcDNA3ORF2+ plasmid and GMCSF+ plasmid;

- second and third injection (simultaneous)

pcDNA3ORF1+ plasmid, pcDNA3ORF2+ plasmid and GMCSF+ plasmid;

Cells transformed by BAC ORF1+ and BAC ORF2+ recombinant baculoviruses capable of respectively expressing the Rep protein of sequence SEQ ID No. 14 and the protein of sequence SEQ ID No. 15 of the PWD circovirus of TYPE B.

Batch D (F4) (control batch): no injection

The batches of piglets B, C and D are infected (tested) at the age of 6 weeks although batch A is not subjected to the test.

3) Observation of the batches

- counting of coughing/sneezing: 15 minutes/batch/day;
- consistency of fecal matter: every day;
- regular recordings: weekly taking of blood, weighing;
- weighing of food refuse: 3 times per week;
- calculation of the daily mean gain in weight (dmg);

The daily mean gains were calculated for each of the batches over a period of 28 days following testing (cf. Figure 10), an intermediate calculation of the dmg was likewise carried out for each of the batches over the first and second periods of 14 days. The results obtained are reported below in Table 6.

Table 6: Daily mean gains

	F1	F2	F3	F4
d0-d14	411 g	450 g	511 g	461 g
d14-d28	623 g	362 g	601 g	443 g
d0-d28	554 g	406 g	556 g	452 g

- Measurement of hyperthermia

The measurement of hyperthermia, of greater than 41°C (cf. Figure 11) and greater than 40.2°C, was carried out for each of the batches over a total period of 28 days following testing. The results obtained, corresponding to the ratio expressed as a percentage between the number of recordings of heat of greater than 41°C (or greater than 40.2°C) and the total number of recordings of heat carried out on all of the pigs per one-week period are reported below in Tables 7 and 8, respectively for the hyperthermia measurements of greater than 41°C and greater than 40.2°C.

Table 7: Hyperthermia > 41°C

	F1	F2	F3	F4
W1	4.1	0.	0.	0.
W2	10.7	16.	0.	8.9
W3	4.7	27.	0.	45.
W4	0.	0.	0.	7.5

Table 8: Hyperthermia > 40.2

	F1	F2	F3	F4
W1	29.1	10.41	29.1	20.8
W2	28.5	39.2	10.7	37.5
W3	14.3	68.7	25.0	81.2
W4	3.3	17.5	20.0	55

4) Conclusion

The recordings carried out clearly show that the animals which received the three injections of a vaccine composition comprising nucleic acid fragments of PWD circovirus according to the invention and/or capable of expressing recombinant proteins of PWD circovirus, in particular of type B, did not exhibit hyperthermia (cf. Figure 10). These animals additionally did not experience a decline in their growth, the dmgs being comparable to those of uninfected control animals (cf. Figure 9). They did not exhibit any particular clinical sign.

These results demonstrate the efficacious protection of the piglets against infection with a PWD circovirus of the invention, the primary agent responsible for PWD or FPW, provided by a vaccine composition prepared from a nucleic acid fragment of the nucleic sequence of PWD circovirus according to the invention, in particular of type B, and/or from recombinant proteins encoded by these nucleic acid fragments.

These results in particular show that the proteins encoded by the ORF1 and ORF2 of PWD circovirus according to the invention are immunogenic proteins inducing an efficacious protective response for the prevention of infection by a PWD circovirus.

EXAMPLE 6: Serological diagnosis of PWD circovirus by immunodetermination using recombinant proteins or synthetic peptides of PWD circovirus

A - Serological diagnosis with recombinant proteins

The identification and the sequencing of porcine PWD circovirus allow recombinant proteins of PWD circovirus to be produced by the techniques of genetic recombination well known to the person skilled in the art.

By these techniques, recombinant proteins encoded, in particular, by the ORF'2 of the PWD circovirus, type B, were expressed by transformed Sf9 insect cells and then isolated.

These recombinant proteins encoded by the ORF'2 are extracted, after culture of the transformed Sf9 cells, by thermal cell lysis by means of 3 cycles of

freezing/thawing to -70°C/+37°C. Healthy Sf9 cells or nontransformed control Sf9 cells are also lyzed.

These two antigenic fractions originating from nontransformed control Sf9 cells and Sf9 cells expressing the ORF'2 are precipitated at 4°C by a 60% plus or minus 5% saturated ammonium sulfate solution. Determination of total proteins is carried out with the aid of the Biorad kit. 500 ng of control Sf9 proteins and of semipurified Sf9 proteins expressing the ORF'2, in solution in 0.05 M bicarbonate buffer pH 9.6, are passively adsorbed at the bottom of 3 different cupules of a Nunc Maxisorp microplate by incubation for one night at +4°C.

The reactivity of pig sera with respect to each of these antigenic fractions is evaluated by an indirect ELISA reaction of which the experimental protocol is detailed below:

- Saturation step: 200 µl/cupule of PBS1X/3% semi-skimmed milk, 1 h 30 incubation at 37°C.
- Washing: 200 µl/cupule of PBS1X/Tween 20: 0.05%, 3 rapid washes.
- Serum incubation step: 100 µl/cupule of serum diluted to 1/100 in PBS1X/semi-skimmed milk, 1%/Tween 20: 0.05%, 1 h incubation at 37°C.
- Washing: 200 µl/cupule of PBS1X/Tween 20: 0.05%, 2 rapid washes followed by 2 washes of 5 min.
- Conjugate incubation step: 50 µl/cupule of rabbit anti-pig conjugate diluted to 1/1000 in PBS1X/semi-skimmed milk, 1%/Tween 20: 0.05%, 1 h incubation at 37°C.
- Washing: 200 µl/cupule of PBS1X/Tween 20: 0.05%, 2 rapid washes followed by 2 washes of 5 min.
- Visualization step: 100 µl/cupule of OPD substrate/citrate buffer/H_2O_2, 15 min incubation at 37°C.
- Stopping of reaction: 50 µl/cupule of 1 N H_2SO_4.
- Reading in a spectrophotometer at 490 nm.

Results

The results obtained are shown below in Table 9.

Table 9

Antigens	Reactivity of Pig Serum not inoculated with Circovirus	Reactivity of Pig Serum inoculated with Circovirus
Purified Sf9 control	0.076	0.088
Sf9 expressing purified ORF'2	0.071	1.035

The results are expressed in optical density measured in a spectrophotometer at 490 nm during analysis by ELISA of the reactivity of pig sera which are or are not inoculated with the type B PWD circovirus according to the protocol indicated above.

B - Serological Diagnosis by Synthetic Peptide

The epitopic mapping of the proteins encoded, for example, by the nucleic sequences ORF1 and ORF2 of the two types of PWD circovirus (types A and B) additionally allowed immunogenic circoviral epitopes to be identified on the proteins encoded by the nucleic sequences ORF'1 and ORF'2 as well as the specific epitopes of the protein encoded by the nucleic sequence ORF'2 of the type B PWD circovirus. Four specific epitopes of the type B PWD circovirus and one epitope common to the two types of PWD circovirus situated on the protein encoded by the nucleic sequence ORF'2 were synthesized in peptide form. The equivalent peptides in the circovirus of type A were likewise synthesized. All these peptides were evaluated as diagnostic antigens within the context of carrying out a serological test.

Results

The results obtained are shown in Table 10 below.

Table 10: Results of the evaluation as a diagnostic antigen of synthetic peptides encoded by the nucleic sequences ORF2 and ORF'2 of PWD circovirus of type A and B.

				Infected pig serum reactivity Circovirus B			
Peptide	**Type PWD circovirus**	**Position**	**AA sequence**	**SPF D0/D54**	**Conventional 1 D0/D42**	**Conventional 2 D0/D42**	**Epitopic specificity**
121	B	71-85	VDMMRFNINDFLPPG	+/-, +++	+/-, +++	-, +++	Circovirus B
177	B	70-84	NVNELRFNIGQFLPP	+/-, +	+/-, +/-	+/-, -	
131	B	115-129	QGDRGVGSSAVILDD	+/-, +/-	++, ++	+/-, +	Circovirus B
188	A	114-127	TSNQRGVGSTVVIL	+/-, -	-, +/-	+/-, +/-	
133	B	119-134	GVGSSAVILDDNVFTK	-, ++	++, +++	+/-, ++	
189	A	118-132	RGVGSTVVILDANFV	+/-, -	-, +/-	+/-, +/-	
146	B	171-185	FTIDYFQPNNKRNQL	-, +/-	-, ++	-, ++	Circovirus A&B
202	A	170-184	DQTIDWFQPNNKRNQ	+++, +++	+/-, ++	+, ++	
152	B	195-209	VDHVGLGTAFENSIY	-, ++	+++, +++	+/-, +	Circovirus B
208	A	194-208	NVEHTGLGYALQNAT	-, -	-, -	-, -	

+/-, +, ++, +++. Increasing intensities of the reactivities observed in Spot peptides on a nitrocellulose membrane. The porcine sera tested are from animals experimentally infected with the circovirus of type B within the animal houses of the CNEVA. Samples are taken from the animals before inoculation on d0 and 42 days or 54 days after inoculation, on d42, d54.

EXAMPLE 7: Characterization of the specific epitopes of the PWD circovirus of type B

The proteins encoded by the ORF2 of the porcine circoviruses of type A and B were chosen for this study. For each of the ORF2s (types A and B), 56 peptides of 15 amino acids which overlap every 4 amino acids were synthesized, thus covering the whole of the protein (cf. Table 11 below).

Table 11: Sequence of amino acids of the 56 peptides of 15 amino acids synthesized from the nucleic sequence ORF'2 (type B) and ORF2 (type A) of PWD circovirus with their corresponding spot number (cf. Figure 12)

Type B ORF'2		Type A ORF2	
Spot No.	Sequence	Spot No.	Sequence
107	HRPRSHLGQILRRRP	163	TRPRSHLGNILRRRP
108	SHLGQILRRRPWLVH	164	SHLGNILRRRPYLVH
109	QILRRRPWLVHPRHR	165	NILRRRPYLVHPAFR
110	RRPWLVHPRHRYRWR	166	RRPYLVHPAFRNRYR
111	LVHPRHRYRWRRKNG	167	LVHPAFRNRYRWRRK
112	RHRYRWRRKNGIFNT	168	AFRNRYRWRRKTGIF
113	RWRRKNGIFNTRLSR	169	RYRWRRKTGIFNSRL
114	KNGIFNTRLSRTFGY	170	RRKTGIFNSRLSREF
115	FNTRLSRTFGYTVKR	171	GIFNSRLSREFVLTI
116	LSRTFGYTVKRTTVR	172	SRLSREFVLTIRGGH
117	FGYTVKRTTVRTPSW	173	REFVLTIRGGHSQPS
118	VKRTTVRTPSWAVDM	174	LTIRGGHSQPSWNVN
119	TVRTPSWAVDMMRFN	175	GGHSQPSWNVNELRF
120	PSWAVDMMRFNINDF	176	QPSWNVNELRFNIGQ
121	VDMMRFNINDFLPPG	177	NVNELRFNIGQFLPP
122	RFNINDFLPPGGGSN	178	LRFNIGQFLPPSGGT
123	NDFLPPGGGSNPRSV	179	IGQFLPPSGGTNPLP
124	PPGGGSNPRSVPFEY	180	LPPSGGTNPLPLPFQ
125	GSNPRSVPFEYYRIR	181	GGTNPLPLPFQYYRI
126	RSVPFEYYRIRKVKV	182	PLPLPFQYYRIRKAK
127	FEYYRIRKVKVEFWP	183	PFQYYRIRKAKYEFY
128	RIRKVKVEFWPCSPI	184	YRIRKAKYEFYPRDP
129	VKVEFWPCSPITQGD	185	KAKYEFYPRDPITSN
130	FWPCSPITQGDRGVG	186	EFYPRDPITSNQRGV
131	SPITQGDRGVGSSAV	187	RDPITSNQRGVGSTV
132	QGDRGVGSSAVILDD	188	TSNQRGVGSTVVILD

133	GVGSSAVILDDNFVT	189	RGVGSTVVILDANFV
134	SAVILDDNFVTKATA	190	STVVILDANFVTPST
135	LDDNFVTKATALTYD	191	ILDANFVTPSTNLAY
136	FVTKATALTYDPYVN	192	NFVTPSTNLAYDPYI
137	ATALTYDPYVNYSSR	193	PSTNLAYDPYINYSS
138	TYDPYVNYSSRHTIT	194	LAYDPYINYSSRHTI
139	YVNYSSRHTITQPFS	195	PYINYSSRHTIRQPF
140	SSRHTITQPFSYHSR	196	YSSRHTIRQPFTYHS
141	TITQPFSYHSRYFTP	197	HTIRQPFTYHSRYFT
142	PFSYHSRYFTPKPVL	198	QPFTYHSRYFTPKPE
143	HSRYFTPKPVLDFTI	199	YHSRYFTPKPELDQT
144	FTPKPVLDFTIDYFQ	200	YFTPKPELDQTIDWF
145	PVLDFTIDYFQPNNK	201	KPELDQTIDWFQPNN
146	FTIDYFQPNNKRNQL	202	DQTIDWFQPNNKRNQ
147	YFQPNNKRNQLWLRL	203	DWFQPNNKRNQLWLH
148	NNKRNQLWLRLQTAG	204	PNNKRNQLWLHLNTH
149	NQLWLRLQTAGNVDH	205	RNQLWLHLNTHTNVE
150	LRLQTAGNVDHVGLG	206	WLHLNTHTNVEHTGL
151	TAGNVDHVGLGTAFE	207	NTHTNVEHTGLGYAL
152	VDHVGLGTAFENSIY	208	NVEHTGLGYALQNAT
153	GLGTAFENSIYDQEY	209	TGLGYALQNATTAQN
154	AFENSIYDQEYNIRV	210	YALQNATTAQNYVVR
155	SIYDQEYNIRVTMYV	211	NATTAQNYVVRLTIY
156	QEYNIRVTMYVQFRE	212	AQNYVVRLTIYVQFR
157	IRVTMYVQFREFNFK	213	VVRLTIYVQFREFIL
158	MYVQFREFNFKDPPL	214	TIYVQFREFILKDPL
159	VQFREFNFKDPPLNP	215	YVQFREFILKDPLNE

These peptides were synthesized according to the "spot" method which consists in simultaneous synthesis of a large number of peptides on a cellulose solid support, each site of synthesis of a peptide constituting a spot (Synt:em, NIMES). This method involves orientation of the peptides on the plate, these being fixed covalently by the carboxy-terminal end. A spot represents approximately 50 nmol of peptide.

The reference of the spots and corresponding peptide sequences is given in Table 11.

These membranes were used for immunoreactivity tests with respect to serum of SPF pigs which were or were not infected experimentally with the type B PWD circoviral strain as well as with respect to sera of infected pigs from conventional farms (conventional farms 1 or 2). This study allowed specific

immunoreactive peptides of the circovirus of type B corresponding to the spots No. 121, No. 132, No. 133 and No. 152 (respectively of amino acid sequences SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19 and SEQ ID No. 20) to be demonstrated. An illustration is shown in Figure 12 where the membranes are visualized with an infected pig serum coming from a conventional farm. Nonspecific immunoreactive peptides of type [lacuna] were likewise demonstrated, among which we shall keep the peptide No. 146 which is strongly immunogenic.

A comparison between the peptide sequences of circoviruses of type A and B (Figure 13) indicates a divergence ranging from 20 to 60% for the specific immunoreactive peptides of the type B, and a weaker divergence (13%) between the nonspecific peptides.

EXAMPLE 8: Protection of Swine From Post-Weaning Multisystemic Wasting Syndrome (PMWS) Conferred by Procine Circovirus Type B (PCV-B) ORF'2 Protein

The ORF'1-encoded protein (REP) and ORF'2-encoded putative capsid protein of PCV-B were expressed, either in insect cells by recombinant baculovirus vectors, or in mammalian cell lines by transfection with plasmidic expression vectors. These two circovirus-derived proteins were detectable in both expression system. As evaluated by weight gains, hyperthermia and absence of lesions following challenge, the pigs were protected against a virulent circovirus challenge after one first DNA immunization with plasmids directing ORF'2 protein and GM-CSF expression and a second injection, 15 days later, with the same plasmid preparation plus the ORF'2 recombinant protein. A lower level of protection was observed when the pigs were vaccinated with ORF'1 protein, as opposed to pigs vaccinated with ORF'2 protein.

A. Development of an experimental model of PMWS in swine:

Eight 3 week-old SPF pigs were inoculated intratracheally (5 ml) and intramuscularly (1 ml).

B. Production and control of PCV-B plasmids:

PCV-B ORF'1 and ORF'2 genes , isolated from PCV-B challenge strain, have been cloned into vector plasmid pcDNA3.1.

All constructs have been validated through a partial sequencing of the PCV-B genes in the final plasmids and expression control by immunoperoxidase on PK15 cells respectively transfected with each plasmid , using swine polyclonal antibodies.

Plasmid encoding GM-CSF has been co-administred.

C. Construction of recombinant baculoviruses:

ORF'1 and ORF'2 proteins were expressed under polyhedrin promoter control. Recombinant proteins were detected by western-blot using swine polyclonal antibodies.

D. Vaccination and challenge:

Four groups of 7 pigs were vaccinated intramuscularly at day 0 (Do), two weeks later, they received the same plasmid preparation plus the recombinant baculovirus.

E. Monitoring:

All groups of pigs were housed in isolated experimental units with air filtration and low air pressure. Clinical observations and rectal temperatures were recorded every day. The pigs were weighed weekly.

F. Conclusions

Expression of PCV-B ORF'2 or PCV-B ORF'1 in swine resulted in a significantly enhanced level of protection as evaluated by weight evolution and body temperature evolution following challenge with PCV-B circovirus. These results are summarized in Figures 14 and 15.

The invention described herein may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The specific embodiments previously described are therefore to be considered as illustrative of,

and not limiting, the scope of the invention. Additionally, the disclosure of all publications and patent applications cited above and below, including International Patent Application No. PCT/FR98/02634, filed December 4, 1998, and published as International Publication No. WO 99/29871 on June 17, 1999, are expressly incorporated herein by reference in their entireties to the same extent as if each were incorporated by reference individually.

Sequence Listing

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<120> GENOMIC AND POLYPEPTIDE CIRCOVIRUS SEQUENCE
ASSOCIATED WITH PIGLET WEIGHT LOSS DISEASE (PWD),
APPLICATIONS TO DIAGNOSIS AND TO THE PREVENTION
AND/OR TO THE TREATMENT OF THE INFECTION

<130> D17221

<140>
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<150> FR 97 15396
<151> 1997-12-05

<160> 20

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Gln Thr Phe Asn Lys Val Lys Trp Tyr Phe Gly Ala Arg Cys His Ile
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Glu Lys Ala Lys Gly Thr Asp Gln Gln Asn Lys Glu Tyr Cys Ser Lys
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Asp Trp Lys Thr Ala Val His Val Ile Val Gly Pro Pro Gly Cys Gly
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Lys Ser Gln Trp Ala Arg Asn Phe Ala Glu Pro Arg Asp Thr Tyr Trp
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Lys Pro Ser Arg Asn Lys Trp Trp Asp Gly Tyr His Gly Glu Glu Val
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Val Val Leu Asp Asp Phe Tyr Gly Trp Leu Pro Trp Asp Asp Leu Leu
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Arg Leu Cys Asp Arg Tyr Pro Leu Thr Val Glu Thr Lys Gly Gly Thr
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Gln Glu Trp Tyr Ser Ser Thr Ala Val Pro Ala Val Glu Ala Leu Tyr
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Arg Arg Ile Thr Thr Leu Gln Phe Trp Lys Thr Ala Gly Glu Gln Ser
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<213> Type A PWD circovirus

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Leu Pro Pro Ser Gly Gly Thr Asn Pro Leu Pro Leu Pro Phe Gln Tyr
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Tyr Arg Ile Arg Lys Ala Lys Tyr Glu Phe Tyr Pro Arg Asp Pro Ile
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Tyr Phe Thr Pro Lys Pro Glu Leu Asp Gln Thr Ile Asp Trp Phe Gln
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Asn Val Glu His Thr Gly Leu Gly Tyr Ala Leu Gln Asn Ala Thr Thr
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    50                  55                  60

Ile Arg Tyr Arg Lys Leu Leu Gly Tyr Ser His Gln Arg Pro Arg Leu
65                  70                  75                  80

Gln Lys Gly Thr His Ser Ser Arg Gln Val Ala Ala Leu Pro Leu Val
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Pro Arg Ser Ser Thr Leu Asp Lys Tyr Val Ala Phe Phe Thr Ala Val
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Phe Val Phe Leu Leu Leu Gly Arg Ile Ile Lys Gly Glu His Pro Pro
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<213> Type B PWD circovirus

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<212> GENOMIC DNA
<213> Type B PWD circovirus

<220> Polarity strand - (5'-3')

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ggcaacttac tgatggagtg tggagctcct agatctcagg gacaacggag tgacctgtct 360
actgctgtga gtaccttgtt ggagagcggg agtctggtga ccgttgcaga gcagcaccct 420
gtaacgtttg tcagaaattt ccgcgggctg gctgaacttt tgaaagtgag cgggaaaatg 480
cagaagcgtg attggaagac taatgtacac gtcattgtgg ggccacctgg gtgtggtaaa 540
agcaaatggg ctgctaattt tgcagacccg gaaaccacat actggaaacc acctagaaac 600
aagtggtggg atggttacca tggtgaagaa gtggttgtta ttgatgactt ttatggctgg 660
ctgccctggg atgatctact gagactgtgt gatcgatatc cattgactgt agagactaaa 720
ggtggaactg taccttttt ggcccgcagt attctgatta ccagcaatca gaccccgttg 780
gaatggtact cctcaactgc tgtcccagct gtagaagctc tttatcggag gattacttcc 840
ttggtatttt ggaagaatgc tacagaacaa tccacggagg aaggggggca gttcgtcacc 900
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<211> 702
<212> DNA
<213> Type B PWD circovirus

<220> ORF2

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aaaaatggca tcttcaacac ccgcctctcc cgcaccttcg gatatactgt caagcgaacc 180
acagtcagaa cgccctcctg ggcggtggac atgatgagat tcaatattaa tgactttctt 240
cccccaggag gggggtcaaa cccccgctct gtgccctttg aatactacag aataagaaag 300
gttaaggttg aattctggcc ctgctccccg atcacccagg gtgacagggg agtgggctcc 360
agtgctgtta ttttagatga taactttgta acaaaggcca cagccctcac ctatgacccc 420
tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480
tttaccccca aacctgtcct agatttcact attgattact tccaaccaaa caacaaaaga 540
aaccagctgt ggctgagact acaaactgct ggaaatgtag accacgtagg cctcggcact 600
gcgttcgaaa acagtatata cgaccaggaa tacaatatcc gtgtaaccat gtatgtacaa 660
ttcagagaat ttaattttaa agacccccca cttaaccctt aa 702

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<213> Type B PWD circovirus

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agtcttccaa tcacgcttct gcattttccc gctcactttc aaaagttcag ccagcccgcg 180
gaaatttctg acaaacgtta cagggtgctg ctctgcaacg gtcaccagac tcccgctctc 240
caacaaggta ctcacagcag tagacaggtc actccgttgt ccctgagatc taggagctcc 300
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<210> 14
<211> 314
<212> PRT
<213> Type B PWD circovirus

<400> 14

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Met Pro Ser Lys Lys Asn Gly Arg Ser Gly Pro Gln Pro His Lys Arg
  1               5                  10                  15

Trp Val Phe Thr Leu Asn Asn Pro Ser Glu Asp Glu Arg Lys Lys Ile
             20                  25                  30

Arg Asp Leu Pro Ile Ser Leu Phe Asp Tyr Phe Ile Val Gly Glu Glu
         35                  40                  45

Gly Asn Glu Glu Gly Arg Thr Pro His Leu Gln Gly Phe Ala Asn Phe
     50                  55                  60

Val Lys Lys Gln Thr Phe Asn Lys Val Lys Trp Tyr Leu Gly Ala Arg
 65                  70                  75                  80

Cys His Ile Glu Lys Ala Lys Gly Thr Asp Gln Gln Asn Lys Glu Tyr
                 85                  90                  95

Cys Ser Lys Glu Gly Asn Leu Leu Met Glu Cys Gly Ala Pro Arg Ser
            100                 105                 110

Gln Gly Gln Arg Ser Asp Leu Ser Thr Ala Val Ser Thr Leu Leu Glu
        115                 120                 125

Ser Gly Ser Leu Val Thr Val Ala Glu Gln His Pro Val Thr Phe Val
    130                 135                 140

Arg Asn Phe Arg Gly Leu Ala Glu Leu Leu Lys Val Ser Gly Lys Met
145                 150                 155                 160

Gln Lys Arg Asp Trp Lys Thr Asn Val His Val Ile Val Gly Pro Pro
                165                 170                 175

Gly Cys Gly Lys Ser Lys Trp Ala Ala Asn Phe Ala Asp Pro Glu Thr
            180                 185                 190

Thr Tyr Trp Lys Pro Pro Arg Asn Lys Trp Trp Asp Gly Tyr His Gly
        195                 200                 205

Glu Glu Val Val Val Ile Asp Asp Phe Tyr Gly Trp Leu Pro Trp Asp
    210                 215                 220
      .

Asp Leu Leu Arg Leu Cys Asp Arg Tyr Pro Leu Thr Val Glu Thr Lys
225                 230                 235                 240

Gly Gly Thr Val Pro Phe Leu Ala Arg Ser Ile Leu Ile Thr Ser Asn
                245                 250                 255

Gln Thr Pro Leu Glu Trp Tyr Ser Ser Thr Ala Val Pro Ala Val Glu
            260                 265                 270

Ala Leu Tyr Arg Arg Ile Thr Ser Leu Val Phe Trp Lys Asn Ala Thr
```

```
        275                 280                 285

Glu Gln Ser Thr Glu Glu Gly Gly Gln Phe Val Thr Leu Ser Pro Pro
    290                 295                 300

Cys Pro Glu Phe Pro Tyr Glu Ile Asn Tyr
305                 310


<210> 15
<211> 233
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<213> Type B PWD circovirus

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Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
  1               5                  10                  15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
             20                  25                  30

Arg His Arg Tyr Arg Trp Arg Arg Lys Asn Gly Ile Phe Asn Thr Arg
         35                  40                  45

Leu Ser Arg Thr Phe Gly Tyr Thr Val Lys Arg Thr Thr Val Arg Thr
     50                  55                  60

Pro Ser Trp Ala Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
 65                  70                  75                  80

Pro Pro Gly Gly Gly Ser Asn Pro Arg Ser Val Pro Phe Glu Tyr Tyr
                 85                  90                  95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100                 105                 110

Gln Gly Asp Arg Gly Val Gly Ser Ser Ala Val Ile Leu Asp Asp Asn
        115                 120                 125

Phe Val Thr Lys Ala Thr Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr
    130                 135                 140

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145                 150                 155                 160

Phe Thr Pro Lys Pro Val Leu Asp Phe Thr Ile Asp Tyr Phe Gln Pro
                165                 170                 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Ala Gly Asn
            180                 185                 190

Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr Asp
        195                 200                 205


Gln Glu Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
    210                 215                 220

Asn Phe Lys Asp Pro Pro Leu Asn Pro
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225 230

<210> 16
<211> 104
<212> PRT
<213> Type B PWD circovirus

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1 5 10 15

Phe Arg Val Cys Lys Ile Ser Ser Pro Phe Ala Phe Thr Thr Pro Arg
20 25 30

Trp Pro His Asn Asp Val Tyr Ile Ser Leu Pro Ile Thr Leu Leu His
35 40 45

Phe Pro Ala His Phe Gln Lys Phe Ser Gln Pro Ala Glu Ile Ser Asp
50 55 60

Lys Arg Tyr Arg Val Leu Leu Cys Asn Gly His Gln Thr Pro Ala Leu
65 70 75 80

Gln Gln Gly Thr His Ser Ser Arg Gln Val Thr Pro Leu Ser Leu Arg
85 90 95

Ser Arg Ser Ser Thr Leu His Gln
100

<210> 17
<211> 15
<212> PRT
<213> Type B PWD circovirus

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1 5 10 15

<210> 18
<211> 15
<212> PRT
<213> Type B PWD circovirus

<400> 18
Gln Gly Asp Arg Gly Val Gly Ser Ser Ala Val Ile Leu Asp Asp
1 5 10 15

<210> 19
<211> 15
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<213> Type B PWD circovirus

<400> 19
Gly Val Gly Ser Ser Ala Val Ile Leu Asp Asp Asn Phe Val Thr
1 5 10 15

<210> 20
<211> 15
<212> PRT
<213> Type B PWD circovirus

<400> 20
Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr
1 5 10 15

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We Claim:

1. A vaccine comprising a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle.

2. A vaccine according to claim 1, wherein the nucleotide sequence is selected from SEQ ID No. 9 or SEQ ID No. 10.

3. A vaccine according to claim 1, wherein the homologue has at least 80% sequence identity to SEQ ID No. 9 or SEQ ID No. 10.

4. A vaccine according to claim 1, wherein the nucleotide sequence is selected from SEQ ID No. 11 or SEQ ID No. 12, or a homologue or fragment thereof.

5. A vaccine according to claim 4, wherein the homologue has at least 80% sequence identity to SEQ ID No. 11 or SEQ ID No. 12.

6. A vaccine according to claim 4, wherein the nucleotide sequence is SEQ ID No. 12.

7. A vaccine comprising a polypeptide encoded by a nucleotide sequence of the genome of PCVB, or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle.

8. A vaccine according to claim 7, wherein the homologue has at least 80% sequence identity to SEQ ID No. 9 or SEQ ID No. 10.

9. A vaccine according to claim 7, wherein the nucleotide sequence is selected from SEQ ID No. 11 or SEQ ID No. 12, or a homologue or fragment thereof.

10. A vaccine according to claim 9, wherein the homologue has at least 80% sequence identity to SEQ ID No. 11 or SEQ ID No. 12.

11. A vaccine according to claim 9, wherein the nucleotide sequence is SEQ ID No. 12.

12. A vaccine according to claim 7, wherein the polypeptide has the amino acid sequence of SEQ ID No. 14 or SEQ ID No. 15.

13. A vaccine according to claim 12, wherein the polypeptide has the amino acid sequence of SEQ ID No. 15.

14. A vaccine according to claim 7, wherein the homologue has at least 80% sequence identity to SEQ ID No. 14 or SEQ ID No. 15.

15. A vaccine according to claim 14, wherein the homologue has at least 80% sequence identity to SEQ ID No. 15.

16. A vaccine according to claim 7, wherein the polypeptide has the amino acid sequence of SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, or SEQ ID No. 20.

17. A vaccine comprising a vector and an acceptable pharmaceutical or veterinary vehicle, the vector comprising a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof.

18. A vaccine according to claim 17, further comprising a gene coding for an expression product capable of inhibiting or retarding the establishment or development of a genetic or acquired disease.

19. A vaccine comprising a cell and an acceptable pharmaceutical or veterinary vehicle, wherein the cell is transformed with a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof.

20. A vaccine according to claim 1, further comprising an adjuvant.

21. A vaccine comprising a pharmaceutically acceptable vehicle and a single polypetide, wherein the single polypeptide consists of SEQ ID No. 15.

22. A method of immunizing a mammal against piglet weight loss disease comprising administering to a mammal an effective amount of the vaccine of any one of claims 1-21.

Abstract of the Invention

The genome sequences and the nucleotide sequences coding for the PWD circovirus polypeptides, such as the circovirus structural and non-strucutral polypeptides, vectors including the sequences, and cells and animals transformed by the vectors are provided. Methods for detecting the nucleic acids or polypeptides, and kits for diagnosing infection by a PWD circovirus, also are provided. Method for selecting compounds capable of modulating the viral infection are further provided. Pharmaceutical, including vaccines, compositions for preventing and/or treating viral infections caused by PWD circovirus and the use of vectors for preventing and/or treating diseases also are provided.

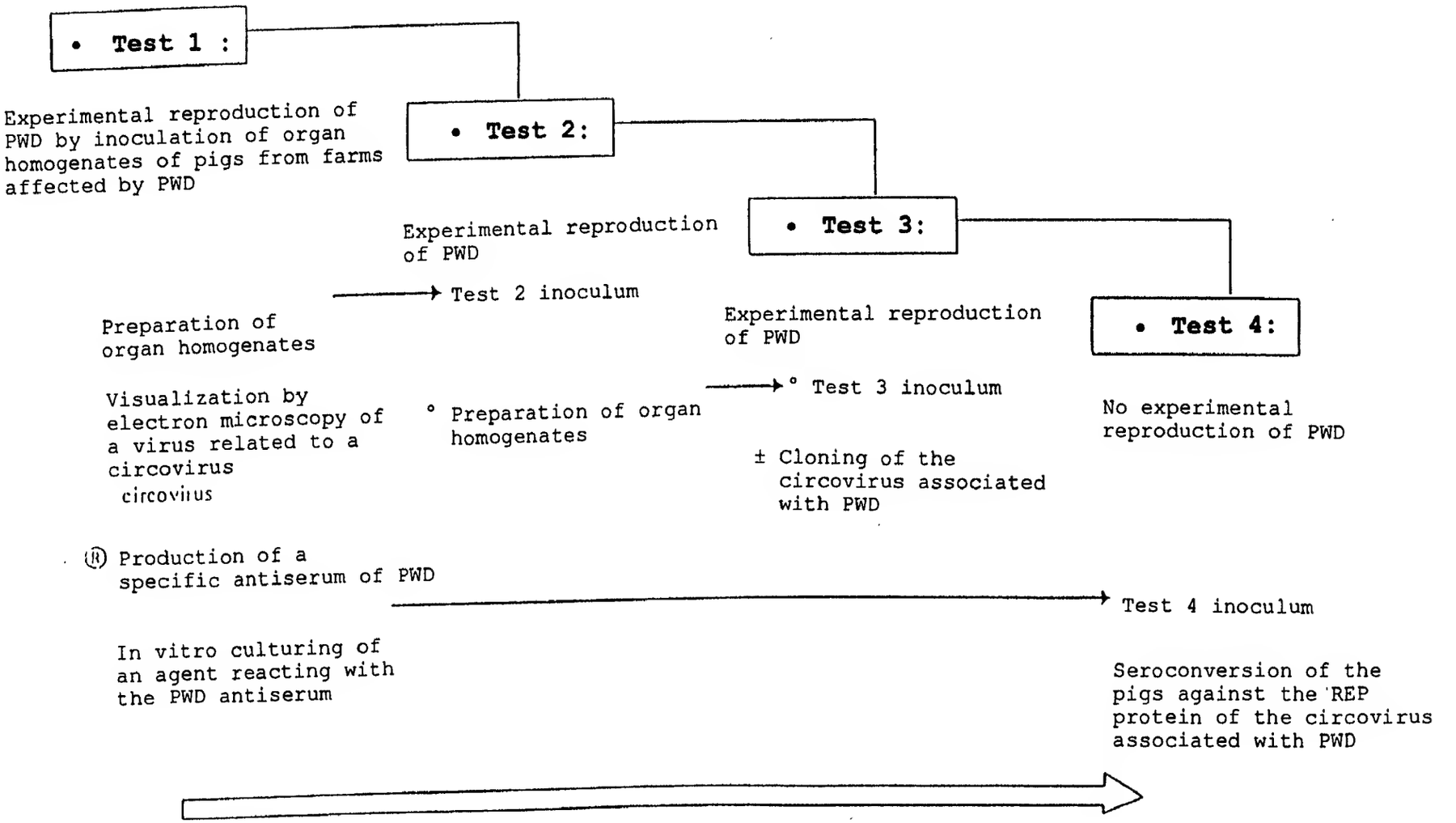

• Test 1 :
Experimental reproduction of PWD by inoculation of organ homogenates of pigs from farms affected by PWD
• Test 2:
Experimental reproduction of PWD
Test 2 inoculum
Preparation of organ homogenates
Visualization by electron microscopy of a virus related to a circovirus
circovirus
° Preparation of organ homogenates
• Test 3:
Experimental reproduction of PWD
° Test 3 inoculum
± Cloning of the circovirus associated with PWD
• Test 4:
No experimental reproduction of PWD
® Production of a specific antiserum of PWD
In vitro culturing of an agent reacting with the PWD antiserum
Test 4 inoculum
Seroconversion of the pigs against the REP protein of the circovirus associated with PWD

FIGURE 1

```
  Leu Ala Ser Arg Cys Arg Cys Cys Arg Pro Leu Thr Leu Ser Phe Ala Leu Cys
 Trp Arg Val Glu Ala Ala Ala Ala Gly Arg Cys Arg *** His Phe His Trp Ala
Gly Ala Cys Lys Pro Leu Pro Leu Val Glu Ala Ala Asp Thr Phe Ile Gly Leu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TGG TCG CGT GAA GCC GTC GCC GTC GTG GAG CCG TCG CAG TCA CTT TTA CGG TTC
          9          18          27          36          45          54
ACC AGC GCA CTT CGG CAG CGG CAG CAC CTC GGC AGC GTC AGT GAA AAT GCC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Ser Ala Leu Arg Gln Arg Gln His Leu Gly Ser Val Ser Glu Asn Ala Lys
 Pro Ala His Phe Gly Ser Gly Ser Thr Ser Ala Ala Ser Val Lys Met Pro Ser
  Gln Arg Thr Ser Ala Ala Ala Ala Pro Arg Gln Arg Gln *** Lys Cys Gln Ala

  Ser Phe Arg Gly Ala Val Gly Tyr Ser Thr Pro Thr *** Gly *** Tyr Asp Lys
 Leu Phe Ala Ala Arg Leu Gly Met Leu Pro Pro His Glu Gly Lys Ile Ile Arg
Leu Phe Leu Pro Gly Cys Gly Trp Leu Leu His Thr Asn Val Arg Leu Leu Gly
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GTT CTT TTC GCC GGG CGT TGG GGT ATT CTC CAC CCA CAA GTG GGA ATT ATT AGG
          63          72          81          90          99         108
CAA GAA AAG CGG CCC GCA ACC CCA TAA GAG GTG GGT GTT CAC CCT TAA TAA TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gln Glu Lys Arg Pro Ala Thr Pro *** Glu Val Gly Val His Pro *** *** Ser
 Lys Lys Ser Gly Pro Gln Pro His Lys Arg Trp Val Phe Thr Leu Asn Asn Pro
  Arg Lys Ala Ala Arg Asn Pro Ile Arg Gly Gly Cys Ser Pro Leu Ile Ile Leu

  Arg Pro Pro Ser Phe Cys Phe Val Pro Ala Glu Leu Arg Gly Lys Gln Asn Asn
 Gly Leu Leu Leu Phe Val Phe Tyr Pro Leu Lys Trp Asp Gly Lys Lys Ile Ile
Glu Ser Ser Ser Phe Phe Leu Ile Arg Ser Ser Gly Ile Glu Arg Lys Ser ***
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAG GCT CCT CCT CTT TTT GTT TTA TGC CCT CGA AGG TTA GAG GGA AAA ACT AAT
        117         126         135         144         153         162
TTC CGA GGA GGA GAA AAA CAA AAT ACG GGA GCT TCC AAT CTC CCT TTT TGA TTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Arg Gly Gly Glu Lys Gln Asn Thr Gly Ala Ser Asn Leu Pro Phe *** Leu
 Ser Glu Glu Glu Lys Asn Lys Ile Arg Glu Leu Pro Ile Ser Leu Phe Asp Tyr
  Pro Arg Arg Arg Lys Thr Lys Tyr Gly Ser Phe Gln Ser Pro Phe Leu Ile Ile

  Gln Lys His Arg Pro Leu Asn Pro Leu Pro Tyr Phe Glu Glu Gly Gly Pro Thr
 Lys Asn Thr Ala Leu Phe Thr Gln Phe Leu Thr Ser Ser Arg Val Glu Leu Pro
Lys Thr Gln Pro Ser Ser Pro Lys Ser Ser Pro Leu Val Gly *** Arg Trp Pro
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAA ACA AAC ACC GCT CCT TCC AAA CCT TCT CCC ATC TTG AGG AGT GGA GGT CCC
        171         180         189         198         207         216
TTT TGT TTG TGG CGA GGA AGG TTT GGA AGA GGG TAG AAC TCC TCA CCT CCA GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Cys Leu Trp Arg Gly Arg Phe Gly Arg Gly *** Asn Ser Ser Pro Pro Gly
 Phe Val Cys Gly Glu Glu Gly Leu Glu Glu Gly Arg Thr Pro His Leu Gln Gly
  Leu Phe Val Ala Arg Lys Val Trp Lys Arg Val Glu Leu Leu Thr Ser Arg Gly

  Gln Ser Asn Gln *** Ser Ala Ser Lys *** Cys Pro Ser Thr Thr Asn Gln His
 Lys Arg Ile Lys Ser Leu Leu Leu Ser Lys Val Leu His Leu Pro Ile Lys Thr
Asn Ala Phe Lys Ala Leu Phe Cys Val Lys Leu Leu Thr Phe His Tyr Lys Pro
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CAA ACG CTT AAA ACG ATT CTT CGT CTG AAA ATT GTT CCA CTT CAC CAT AAA ACC
        225         234         243         252         261         270
GTT TGC GAA TTT TGC TAA GAA GCA GAC TTT TAA CAA GGT GAA GTG GTA TTT TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Cys Glu Phe Cys *** Glu Ala Asp Phe *** Gln Gly Glu Val Val Phe Trp
 Phe Ala Asn Phe Ala Lys Lys Gln Thr Phe Asn Lys Val Lys Trp Tyr Phe Gly
  Leu Arg Ile Leu Leu Arg Ser Arg Leu Leu Thr Arg *** Ser Gly Ile Leu Val
```

FIGURE 2

```
  Gly Ser Gly Cys Arg Ser Leu Ser Leu Phe Arg Gly Ala Ser Tyr Leu Ile Ser
 Gly Ala Ala Val Asp Leu Phe Arg Phe Ser Gly Val Leu Leu Ile Phe Phe Val
Ala Arg Gln Trp Met Ser Phe Ala Phe Pro Val Ser Trp Cys Phe Leu Ser Tyr
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ACG GGC GAC GGT GTA GCT CTT TCG CTT TCC TTG GCT GGT CGT CTT ATT TCT TAT
        279         288         297         306         315         324
TGC CCG CTG CCA CAT CGA GAA AGC GAA AGG AAC CGA CCA GCA GAA TAA AGA ATA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Cys Pro Leu Pro His Arg Glu Ser Glu Arg Asn Arg Pro Ala Glu *** Arg Ile
 Ala Arg Cys His Ile Glu Lys Ala Lys Gly Thr Asp Gln Gln Asn Lys Glu Tyr
  Pro Ala Ala Thr Ser Arg Lys Arg Lys Glu Pro Thr Ser Arg Ile Lys Asn Thr

 Cys Tyr Leu Leu Gly Cys Val *** Arg Thr His Leu Glu Ala Ser Gly Pro Ser
 Ala Thr Phe Phe Ala Val Tyr Lys Asp Leu Thr Ser Ser Arg Pro Val Leu Pro
Gln Leu Leu Ser Pro Trp Met Ser Ile Ser His Pro Ala Gly Arg Phe Trp Pro
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GAC GTC ATT TCT TCC GGT GTA TGA ATA GCT CAC ACC TCG AGG CGC CTT GGT CCC
        333         342         351         360         369         378
CTG CAG TAA AGA AGG CCA CAT ACT TAT CGA GTG TGG AGC TCC GCG GAA CCA GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Gln *** Arg Arg Pro His Thr Tyr Arg Val Trp Ser Ser Ala Glu Pro Gly
 Cys Ser Lys Glu Gly His Ile Leu Ile Glu Cys Gly Ala Pro Arg Asn Gln Gly
  Ala Val Lys Lys Ala Thr Tyr Leu Ser Ser Val Glu Leu Arg Gly Thr Arg Gly

 Ala Cys Arg Gly Thr *** Gln Gln Ser Tyr Gly Lys Pro Ser Pro Thr Lys Pro
 Leu Ala Ala Val Gln Arg Ser Ser His Thr Gly Lys Gln Leu Arg Pro Arg Gln
Phe Arg Leu Ser Arg Asp Val Ala Thr Leu Val Arg Lys Ser Val Pro Asp Lys
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CTT CGC GTC GCT GGA CAG ATG ACG ACA CTC ATG GGA AAA CCT CTG CCC CAG AAA
        387         396         405         414         423         432
GAA GCG CAG CGA CCT GTC TAC TGC TGT GAG TAC CCT TTT GGA GAC GGG GTC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Ala Gln Arg Pro Val Tyr Cys Cys Glu Tyr Pro Phe Gly Asp Gly Val Phe
 Lys Arg Ser Asp Leu Ser Thr Ala Val Ser Thr Leu Leu Glu Thr Gly Ser Leu
  Ser Ala Ala Thr Cys Leu Leu Leu *** Val Pro Phe Trp Arg Arg Gly Leu Trp

  Ser Gln Leu Arg Ala Thr Glu Gln Leu Thr His Ser Phe Asn Gly Arg Ala Pro
 His Ser Tyr Gly Leu Leu Lys Arg Tyr Arg Ile His Ser Ile Glu Ala Pro Gln
Thr Val Thr Ala Ser Cys Asn Gly Thr Val Tyr Thr Leu Phe Lys Arg Pro Ser
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CCA CTG ACA TCG GCT CGT CAA AGG ACA TTG CAT ACA CTC TTT AAA GGC GCC CGA
        441         450         459         468         477         486
GGT GAC TGT AGC CGA GCA GTT TCC TGT AAC GTA TGT GAG AAA TTT CCG CGG GCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Asp Cys Ser Arg Ala Val Ser Cys Asn Val Cys Glu Lys Phe Pro Arg Ala
 Val Thr Val Ala Glu Gln Phe Pro Val Thr Tyr Val Arg Asn Phe Arg Gly Leu
  *** Leu *** Pro Ser Ser Phe Leu *** Arg Met *** Glu Ile Ser Ala Gly Trp

  Gln Val Lys Ser Leu Ser Arg Ser Ser Ala Ala Ala His Asn Ser Ser Leu Gln
 Ser Phe Lys Gln Phe His Ala Pro Leu His Leu Leu Thr Ile Pro Leu Cys Ser
Ala Ser Ser Lys Phe Thr Leu Pro Phe Ile Cys Cys Arg Ser Gln Phe Val Ala
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CCG ACT TGA AAA CTT TCA CTC GCC CTT CTA CGT CGT CGC ACT AAC CTT CTG TCG
        495         504         513         522         531         540
GGC TGA ACT TTT GAA AGT GAG CGG GAA GAT GCA GCA GCG TGA TTG GAA GAC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly *** Thr Phe Glu Ser Glu Arg Glu Asp Ala Ala Ala *** Leu Glu Asp Ser
 Ala Glu Leu Leu Lys Val Ser Gly Lys Met Gln Gln Arg Asp Trp Lys Thr Ala
  Leu Asn Phe *** Lys *** Ala Gly Arg Cys Ser Ser Val Ile Gly Arg Gln Leu
```

FIGURE 2 (continuation 1)

```
  Val Arg *** Leu Pro Gly Ala Arg Asn His Ser Ser Gly Thr Pro Gly Tyr Asn
 Tyr Val Asp Tyr His Ala Arg Gly Thr Thr Pro Leu Ala Leu Pro Gly Thr Ile
Thr Cys Thr Met Thr Pro Gly Gly Pro Gln Pro Phe Leu Trp His Ala Arg Leu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ACA TGT GCA GTA TCA CCC GGG CGG GCC AAC ACC CTT CTC GGT CAC CCG GGC ATT
        549         558         567         576         585         594
TGT ACA CGT CAT AGT GGG CCC GCC CGG TTG TGG GAA GAG CCA GTG GGC CCG TAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Cys Thr Arg His Ser Gly Pro Ala Arg Leu Trp Glu Glu Pro Val Gly Pro ***
 Val His Val Ile Val Gly Pro Pro Gly Cys Gly Lys Ser Gln Trp Ala Arg Asn
  Tyr Thr Ser *** Trp Ala Arg Pro Val Val Gly Arg Ala Ser Gly Pro Val Ile

  Gln Gln Ala *** Pro Cys Arg Ser Ser Ala *** Tyr Phe Tyr Thr Thr Pro His
 Lys Ser Leu Arg Pro Val Gly Val Pro Leu Arg Thr Ser Ile Leu Pro Pro Ile
Lys Ala Ser Gly Leu Ser Val *** Gln Phe Gly Leu Leu Phe Leu His His Ser
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAA ACG ACT CGG ATC CCT GTG GAT GAC CTT CGG ATC ATC TTT ATT CAC CAC CCT
        603         612         621         630         639         648
TTT TGC TGA GCC TAG GGA CAC CTA CTG GAA GCC TAG TAG AAA TAA GTG GTG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Cys *** Ala *** Gly His Leu Leu Glu Ala *** *** Lys *** Val Val Gly
 Phe Ala Glu Pro Arg Asp Thr Tyr Trp Lys Pro Ser Arg Asn Lys Trp Trp Asp
  Leu Leu Ser Leu Gly Thr Pro Thr Gly Ser Leu Val Glu Ile Ser Gly Gly Met

  Ile Asp His Leu Leu Leu Gln Gln Lys Pro His Asn Lys His Ser Thr Val Lys
 Ser Ile Met Ser Phe Phe Asn Asn Asn Gln Ile Ile Lys Ile Ala Pro *** Arg
Pro Tyr *** Pro Ser Ser Thr Thr Thr Lys Ser Ser Lys *** Pro Gln Asn Gly
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ACC TAT AGT ACC TCT TCT TCA ACA ACA AAA CCT ACT AAA AAT ACC GAC CAA TGG
        657         666         675         684         693         702
TGG ATA TCA TGG AGA AGA AGT TGT TGT TTT GGA TGA TTT TTA TGG CTG GTT ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Trp Ile Ser Trp Arg Arg Ser Cys Cys Phe Gly *** Phe Leu Trp Leu Val Thr
 Gly Tyr His Gly Glu Glu Val Val Val Leu Asp Asp Phe Tyr Gly Trp Leu Pro
  Asp Ile Met Glu Lys Lys Leu Leu Phe Trp Met Ile Phe Met Ala Gly Tyr Leu

  Pro His Asp Val Ser Val Thr His Gly Thr Asp Met Ser Gln Leu Ser *** Leu
 Pro Ile Ile *** Gln Ser Gln Thr Val Pro Ile Trp Gln Ser Tyr Leu Ser Phe
Gln Ser Ser Arg Ser Leu Ser His Ser Arg Tyr Gly Asn Val Thr Ser Val Leu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAC CCT ACT AGA TGA CTC TGA CAC ACT GGC CAT AGG TAA CTG ACA TCT CTG ATT
        711         720         729         738         747         756
TTG GGA TGA TCT ACT GAG ACT GTG TGA CCG GTA TCC ATT GAC TGT AGA GAC TAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Gly *** Ser Thr Glu Thr Val *** Pro Val Ser Ile Asp Cys Arg Asp ***
 Trp Asp Asp Leu Leu Arg Leu Cys Asp Arg Tyr Pro Leu Thr Val Glu Thr Lys
  Gly Met Ile Tyr *** Asp Cys Val Thr Gly Ile His *** Leu *** Arg Leu Lys

  Pro Tyr Gln Glu Lys Lys Pro Gly Cys Tyr Lys Ser *** Trp Cys Asp Pro Gly
 Pro Thr Ser Asn Arg Lys Gln Gly Ala Thr Asn Gln Asn Gly Ala Ile Leu Gly
Pro Pro Val Thr Gly Lys Lys Ala Arg Leu Ile Lys Ile Val Leu Leu *** Ala
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TCC CCC ATG ACA AGG AAA AAA CCG GGC GTC ATA AAA CTA ATG GTC GTT AGT CCG
        765         774         783         792         801         810
AGG GGG TAC TGT TCC TTT TTT GGC CCG CAG TAT TTT GAT TAC CAG CAA TCA GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Gly Tyr Cys Ser Phe Phe Gly Pro Gln Tyr Phe Asp Tyr Gln Gln Ser Gly
 Gly Gly Thr Val Pro Phe Leu Ala Arg Ser Ile Leu Ile Thr Ser Asn Gln Ala
  Gly Val Leu Phe Leu Phe Trp Pro Ala Val Phe *** Leu Pro Ala Ile Arg Pro
```

FIGURE 2 (continuation 2)

```
  Gly Pro Ile Thr Ser Arg Leu Gln Gln Gly Leu Gln Leu Leu Glu Arg Asp Ser
 Gly Leu Phe Pro Val Gly *** Ser Ser Asp Trp Ser Tyr Phe Ser Glu Ile Pro
Gly Trp Ser His Tyr Glu Glu Val Ala Thr Gly Ala Thr Ser Ala Arg *** Arg
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GGG GGT CCT TAC CAT GAG GAG TTG ACG ACA GGG TCG ACA TCT TCG AGA GAT AGC
        819         828         837         846         855         864
CCC CCA GGA ATG GTA CTC CTC AAC TGC TGT CCC AGC TGT AGA AGC TCT CTA TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Pro Gly Met Val Leu Leu Asn Cys Cys Pro Ser Cys Arg Ser Ser Leu Ser
 Pro Gln Glu Trp Tyr Ser Ser Thr Ala Val Pro Ala Val Glu Ala Leu Tyr Arg
  Pro Arg Asn Gly Thr Pro Gln Leu Leu Ser Gln Leu *** Lys Leu Ser Ile Gly

  Ser *** *** Lys Ala Ile Lys Ser Ser Gln Gln Leu Val Ile Trp Pro Pro Val
 Pro Asn Ser Ser Gln Leu Lys Pro Leu Ser Ser Ser Phe Leu Gly Arg Leu Tyr
Leu Ile Val Val Lys Cys Asn Gln Phe Val Ala Pro Ser Cys Asp Val Ser Thr
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CTC CTA ATG ATG AAA CGT TAA AAC CTT CTG ACG ACC TCT TGT TAG GTG CCT CCA
        873         882         891         900         909         918
GAG GAT TAC TAC TTT GCA ATT TTG GAA GAC TGC TGG AGA ACA ATC CAC GGA GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Asp Tyr Tyr Phe Ala Ile Leu Glu Asp Cys Trp Arg Thr Ile His Gly Gly
 Arg Ile Thr Thr Leu Gln Phe Trp Lys Thr Ala Gly Glu Gln Ser Thr Glu Val
  Gly Leu Leu Leu Cys Asn Phe Gly Arg Leu Leu Glu Asn Asn Pro Arg Arg Tyr

  Arg Leu Gly Ile Gln Leu Leu Pro Gly Val Arg His Gly Lys Gly Met Tyr Phe
 Gly Phe Ala Ser Lys Phe Cys His Val Trp Gly Thr Gly Lys Glu Trp Ile Phe
Gly Ser Pro Arg Asn Ser Ala Thr Ser Gly Gly Gln Ala Arg Lys Gly Tyr Leu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TGG GCT TCC GGC TAA ACT TCG TCA CCT GGG TGG GAC ACG GGA AAA GGG TAT ATT
        927         936         945         954         963         972
ACC CGA AGG CCG ATT TGA AGC AGT GGA CCC ACC CTG TGC CCT TTT CCC ATA TAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Arg Arg Pro Ile *** Ser Ser Gly Pro Thr Leu Cys Pro Phe Pro Ile ***
 Pro Glu Gly Arg Phe Glu Ala Val Asp Pro Pro Cys Ala Leu Phe Pro Tyr Lys
  Pro Lys Ala Asp Leu Lys Gln Trp Thr His Pro Val Pro Phe Ser His Ile Lys

  Leu Asn Ser Leu Arg Lys Gln *** *** Met Thr Ile Thr Lys Ile Lys Ile ***
 Tyr Ile Val Ser Asp Lys Lys Asn Asp Cys Arg Leu Pro Lys *** Lys *** Glu
Ile Phe *** Gln Thr Lys Lys Thr Ile Val Asp Tyr His Asn Lys Asn Lys Asn
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TTA TTT AAT GAC TCA GAA AAA ACA ATA GTG TAG CAT TAC CAA AAA TAA AAA TAA
        981         990         999        1008        1017        1026
AAT AAA TTA CTG AGT CTT TTT TGT TAT CAC ATC GTA ATG GTT TTT ATT TTT ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Lys Leu Leu Ser Leu Phe Cys Tyr His Ile Val Met Val Phe Ile Phe Ile
 Ile Asn Tyr *** Val Phe Phe Val Ile Thr Ser *** Trp Phe Leu Phe Leu Phe
  *** Ile Thr Glu Ser Phe Leu Leu Ser His Arg Asn Gly Phe Tyr Phe Tyr Ser

  Lys Ser Pro Arg Glu Pro Tyr Ile Arg Gln Ile Thr Cys Leu Tyr Asp Val Lys
 Asn Leu Pro Asp Lys Leu Ile Phe Glu Arg Phe Gln Val Tyr Ile Thr Leu Arg
Met *** Leu Thr Lys *** Ser Leu Asn Glu Ser Asn Tyr Met Phe Leu *** Gly
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GTA AAT CTC CCA GAA AGT CCT ATT TAA GAG ACT TAA CAT GTA TTT ATC AGT TGG
       1035        1044        1053        1062        1071        1080
CAT TTA GAG GGT CTT TCA GGA TAA ATT CTC TGA ATT GTA CAT AAA TAG TCA ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Leu Glu Gly Leu Ser Gly *** Ile Leu *** Ile Val His Lys *** Ser Thr
 Ile *** Arg Val Phe Gln Asp Lys Phe Ser Glu Leu Tyr Ile Asn Ser Gln Pro
  Phe Arg Gly Ser Phe Arg Ile Asn Ser Leu Asn Cys Thr *** Ile Val Asn Leu
```

FIGURE 2 (continuation 3)

```
  Gly Cys Leu Lys Pro Ser His Asn Cys Lys Pro Ala Cys Leu Gly Pro Arg His
 Val Val Tyr Asn Gln Ala Thr Thr Ala Asn Gln Leu Ala Tyr Gly Leu Gly Thr
*** Trp Met Ile Lys Pro Gln Pro Gln Met Lys Ser Arg Met Ala Trp Ala Gln
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAT GGT GTA TTA AAA CCC GAC ACC AAC GTA AAA CCT CGC GTA TCG GGT CCG GAC
       1089        1098        1107        1116        1125        1134
TTA CCA CAT AAT TTT GGG CTG TGG TTG CAT TTT GGA GCG CAT AGC CCA GGC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Pro His Asn Phe Gly Leu Trp Leu His Phe Gly Ala His Ser Pro Gly Leu
 Tyr His Ile Ile Leu Gly Cys Gly Cys Ile Leu Glu Arg Ile Ala Gln Ala Cys
  Thr Thr *** Phe Trp Ala Val Val Ala Phe Trp Ser Ala *** Pro Arg Pro Val

  Ala Arg Cys Gln His Pro Tyr Lys Phe Pro Ala Val Ala Pro Lys Lys *** ***
 His Glu Val Asn Thr His Thr Asn Leu His Leu Trp Leu Gln Asn Arg Lys Asn
Thr Ser Ser Met Pro Thr Pro Ile *** Ile Ser Gly Cys Ser Thr Glu Lys Ile
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ACA CGA GCT GTA ACC ACA CCC ATA AAT TTA CCT CGG TGT CGA CCA AAG AAA ATA
       1143        1152        1161        1170        1179        1188
TGT GCT CGA CAT TGG TGT GGG TAT TTA AAT GGA GCC ACA GCT GGT TTC TTT TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Cys Ala Arg His Trp Cys Gly Tyr Leu Asn Gly Ala Thr Ala Gly Phe Phe Tyr
 Val Leu Asp Ile Gly Val Gly Ile *** Met Glu Pro Gln Leu Val Ser Phe Ile
  Cys Ser Thr Leu Val Trp Val Phe Lys Trp Ser His Ser Trp Phe Leu Leu Leu

  Lys Ala Pro Val Leu *** Asn Asn Pro Arg Ala Arg Thr Gln Pro His Leu Val
 Asn Pro Gln Phe Trp Asp Ile Thr Gln Asp Leu Glu Pro Lys Pro Thr Phe Tyr
Ile Gln Ser Ser Gly Ile Leu Gln Lys Thr *** Ser Gln Asn Pro Pro Ser Thr
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ATA AAC CGA CCT TGG TTA GTT AAC AAA CCA GAT CGA GAC CAA ACC CCC ACT TCA
       1197        1206        1215        1224        1233        1242
TAT TTG GCT GGA ACC AAT CAA TTG TTT GGT CTA GCT CTG GTT TGG GGG TGA AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Leu Ala Gly Thr Asn Gln Leu Phe Gly Leu Ala Leu Val Trp Gly *** Ser
 Ile Trp Leu Glu Pro Ile Asn Cys Leu Val *** Leu Trp Phe Gly Gly Glu Val
  Phe Gly Trp Asn Gln Ser Ile Val Trp Ser Ser Ser Gly Leu Gly Val Lys Tyr

  Gln Leu Pro Leu Tyr Leu Ala Ala Lys His His Pro Pro Leu Leu Leu *** Tyr
 Arg Ser His Tyr Thr Phe Pro Gln Arg Ile Thr His Arg Ser Ser Tyr Asn Ile
Gly Pro Thr Thr Pro Leu Pro Ser Gly *** Pro Thr Ala Pro Pro Thr Thr Leu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TGG ACC TCA CCA TCC ATT TCC CGA CGG AAT ACC ACA CCG CCC TCC TCA TCA ATT
       1251        1260        1269        1278        1287        1296
ACC TGG AGT GGT AGG TAA AGG GCT GCC TTA TGG TGT GGC GGG AGG AGT AGT TAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Trp Ser Gly Arg *** Arg Ala Ala Leu Trp Cys Gly Gly Arg Ser Ser ***
 Pro Gly Val Val Gly Lys Gly Leu Pro Tyr Gly Val Ala Gly Gly Val Val Asn
  Leu Glu Trp *** Val Lys Gly Cys Leu Met Val Trp Arg Glu Glu *** Leu Ile

  Leu Pro *** Leu Gly Leu Gln His Leu Pro Asn Cys Leu Gln Cys Gly Leu Tyr
 Tyr Pro Asp Tyr Ala Leu Asn Thr Ser Pro Thr Val Phe Asn Ala Asp Leu Ile
Ile Pro Thr Met Pro Trp Thr Pro Pro Pro Pro *** Leu Thr Pro Met Trp Ser
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ATA TCC CCA GTA TCC GGT TCA ACC ACC TCC CCC AAT GTT TCA ACC GTA GGT TCT
       1305        1314        1323        1332        1341        1350
TAT AGG GGT CAT AGG CCA AGT TGG TGG AGG GGG TTA CAA AGT TGG CAT CCA AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Arg Gly His Arg Pro Ser Trp Trp Arg Gly Leu Gln Ser Trp His Pro Arg
 Ile Gly Val Ile Gly Gln Val Gly Gly Gly Gly Tyr Lys Val Gly Ile Gln Asp
  *** Gly Ser *** Ala Lys Leu Val Glu Gly Val Thr Lys Leu Ala Ser Lys Ile
```

FIGURE 2 (continuation 4)

```
 Cys Cys His Val Trp Cys Arg Lys Ser *** Leu His His Pro Arg Gln Pro Leu
 Val Val Thr Ser Gly Val Gly Arg Gln Asn Ser Thr Ile Pro Asp Arg Pro Tyr
Leu Leu Leu Pro Gly Leu Val Glu Lys Ile Leu Pro Ser Pro Thr Glu Pro Thr
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ATT GTT GTC ACC TGG GTT GTG GAG AAA CTA ATC TCC ACT ACC CCA GAG ACC CCA
       1359        1368        1377        1386        1395        1404
TAA CAA CAG TGG ACC CAA CAC CTC TTT GAT TAG AGG TGA TGG GGT CTC TGG GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
*** Gln Gln Trp Thr Gln His Leu Phe Asp *** Arg *** Trp Gly Leu Trp Gly
 Asn Asn Ser Gly Pro Asn Thr Ser Leu Ile Arg Gly Asp Gly Val Ser Gly Val
  Thr Thr Val Asp Pro Thr Pro Leu *** Leu Glu Val Met Gly Ser Leu Gly ***

 Ile *** Ile *** Gly Lys *** Tyr Pro Leu Ile Pro Phe Thr Pro Thr Pro Pro
 Phe Glu Tyr Lys Ala Lys Arg Ile Arg Tyr Tyr Gln Phe Pro Leu Pro Leu Pro
Phe Asn Met Asn Leu Arg Glu Leu Val Thr Thr Asn Ser Leu Tyr Pro Tyr Pro
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TTT TAA GTA TAA ATC GGA AAG ATT ATG CCA TCA TAA CCT TTC CAT CCC CAT CCC
       1413        1422        1431        1440        1449        1458
AAA ATT CAT ATT TAG CCT TTC TAA TAC GGT AGT ATT GGA AAG GTA GGG GTA GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Ile His Ile *** Pro Phe *** Tyr Gly Ser Ile Gly Lys Val Gly Val Gly
 Lys Phe Ile Phe Ser Leu Ser Asn Thr Val Val Leu Glu Arg *** Gly *** Gly
  Asn Ser Tyr Leu Ala Phe Leu Ile Arg *** Tyr Trp Lys Gly Arg Gly Arg Gly

 Gln His Arg Arg Leu Pro Pro Pro Val Pro Arg His Gln Ile Glu Ala Arg ***
 Asn Thr Gly Gly Ser Pro Pro Leu Phe Gln Gly Ile Asn Phe Arg Leu Glu Asn
Thr Pro Ala Ala Gln Pro Pro Ser Ser Ser Ala Ser Thr Ser Asp *** Ser Thr
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CCA ACC ACG GCG GAC TCC CCC CCT CCT TGA CCG GCT ACA ACT TAG AGT CGA GCA
       1467        1476        1485        1494        1503        1512
GGT TGG TGC CGC CTG AGG GGG GGA GGA ACT GGC CGA TGT TGA ATC TCA GCT CGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Trp Cys Arg Leu Arg Gly Gly Gly Thr Gly Arg Cys *** Ile Ser Ala Arg
 Val Gly Ala Ala *** Gly Gly Glu Glu Leu Ala Asp Val Glu Ser Gln Leu Val
  Leu Val Pro Pro Glu Gly Gly Arg Asn Trp Pro Met Leu Asn Leu Ser Ser Leu

 Cys Glu Leu Ile Ala Ala Leu Thr Arg Arg Lys His His Thr Cys Ile Arg ***
 Val Asn Trp Ser Pro Gln Ser His Gly Gly Arg Ile Thr Leu Val Phe Glu Arg
Leu Met Gly Leu His Ser Arg Thr Asp Glu Glu *** Pro Ser Tyr Leu Asn Glu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ATT GTA AGG TTC TAC CGA CGC TCA CAG GAG GAG AAT ACC ACT CAT GTT TAA GAG
       1521        1530        1539        1548        1557        1566
TAA CAT TCC AAG ATG GCT GCG AGT GTC CTC CTC TTA TGG TGA GTA CAA ATT CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
*** His Ser Lys Met Ala Ala Ser Val Leu Leu Leu Trp *** Val Gln Ile Leu
 Asn Ile Pro Arg Trp Leu Arg Val Ser Ser Ser Tyr Gly Glu Tyr Lys Phe Ser
  Thr Phe Gln Asp Gly Cys Glu Cys Pro Pro Leu Met Val Ser Thr Asn Ser Leu

 Phe Pro Pro Phe Gln Leu Tyr Gly Asp Lys Pro Ala Met Gln Leu Pro Lys Gln
 Ser Leu Arg Ser Asn Phe Ile Gly Thr Lys Arg Arg Trp Arg Tyr Arg Asn Arg
Leu Phe Ala Pro Ile Ser Ser Val Arg Arg Glu Ala Gly Asp Thr Val Thr Glu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ATC TTT CCG CCC TTA ACT TCT ATG GGC AGA AAG CCG CGG TAG ACA TTG CCA AAG
       1575        1584        1593        1602        1611        1620
TAG AAA GGC GGG AAT TGA AGA TAC CCG TCT TTC GGC GCC ATC TGT AAC GGT TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
*** Lys Gly Gly Asn *** Arg Tyr Pro Ser Phe Gly Ala Ile Cys Asn Gly Phe
 Arg Lys Ala Gly Ile Glu Asp Thr Arg Leu Ser Ala Pro Ser Val Thr Val Ser
  Glu Arg Arg Glu Leu Lys Ile Pro Val Phe Arg Arg His Leu *** Arg Phe Leu
```

FIGURE 2 (continuation 5)

```
 Leu Arg Pro Thr Gly Phe Ile Thr Lys Glu Pro Pro His Lys Trp Ser Pro Gln
 Phe Ala Pro His Val Leu Tyr Pro Arg Arg Arg Leu Ile Asn Gly Leu His Ser
Ser Pro Pro Thr Tyr Trp Ile His Asp Glu Gly Ser Ser Thr Glu Leu Ile Ala
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ACT TCC GCC CCA CAT GGT TTA TAC CAG AAG AGG CCT CCT ACA AAG GTT CTA CCG
       1629        1638        1647        1656        1665        1674
TGA AGG CGG GGT GTA CCA AAT ATG GTC TTC TCC GGA GGA TGT TTC CAA GAT GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
*** Arg Arg Gly Val Pro Asn Met Val Phe Ser Gly Gly Cys Phe Gln Asp Gly
 Glu Gly Gly Val Tyr Gln Ile Trp Ser Ser Pro Glu Asp Val Ser Lys Met Ala
  Lys Ala Gly Cys Thr Lys Tyr Gly Leu Leu Arg Arg Met Phe Pro Arg Trp Leu

  Pro Pro Pro Asp Thr Lys Gln Pro Leu Ala Glu Lys Ala Val Asp Asp *** Leu
 Arg Pro Arg Thr Arg Arg Arg Arg Tyr Arg Arg Arg Pro Trp Thr Met Arg Tyr
Ala Pro Ala Pro Gly Asp Glu Ala Thr Val Gly Gly Gln Gly Arg *** Gly Ile
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ACG CCC CCG CCC AGG CAG AAG ACG CCA TTG CGG AGG AAC CGG TGC AGT AGG ATA
       1683        1692        1701        1710        1719        1728
TGC GGG GGC GGG TCC GTC TTC TGC GGT AAC GCC TCC TTG GCC ACG TCA TCC TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Cys Gly Gly Gly Ser Val Phe Cys Gly Asn Ala Ser Leu Ala Thr Ser Ser Tyr
 Ala Gly Ala Gly Pro Ser Ser Ala Val Thr Pro Pro Trp Pro Arg His Pro Ile
  Arg Gly Arg Val Arg Leu Leu Arg *** Arg Leu Leu Gly His Val Ile Leu ***

  Leu Ser Leu Leu Ala Ser Ser Tyr Tyr
 Phe His Phe Phe His Ala Ala Thr Thr Asn
Phe Thr Phe Ser Thr Arg Gln Gln Leu Ile
--- --- --- --- --- --- --- --- --- --- -
TTT TCA CTT TCT TCA CGC GAC GAC ATC ATA A 5'
       1737        1746        1755
AAA AGT GAA AGA AGT GCG CTG CTG TAG TAT T 3'
--- --- --- --- --- --- --- --- --- --- -
Lys Ser Glu Arg Ser Ala Leu Leu *** Tyr
 Lys Val Lys Glu Val Arg Cys Cys Ser Ile
  Lys *** Lys Lys Cys Ala Ala Val Val
```

FIGURE 2 (continuation 6)

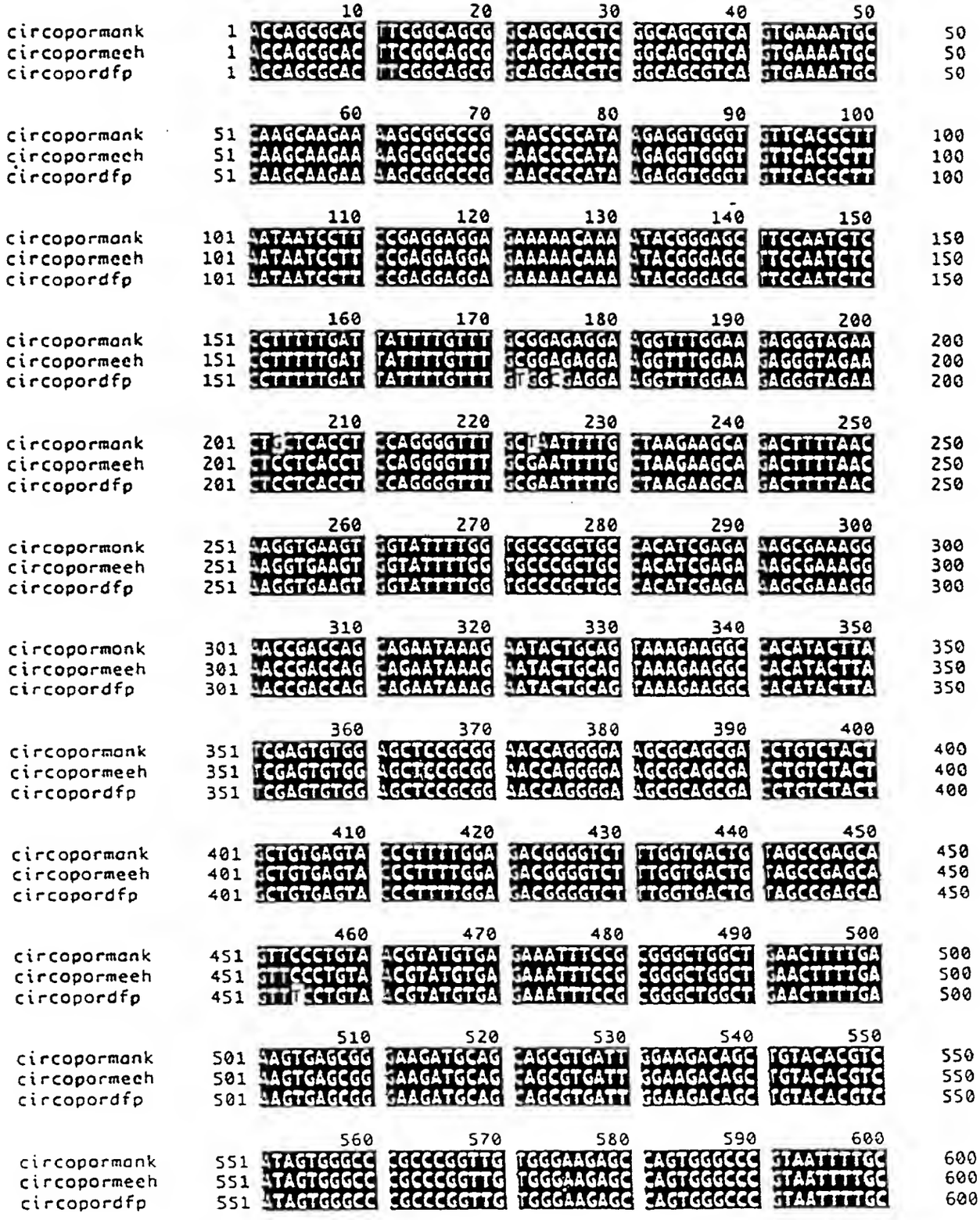

10 20 30 40 50
circopormank 1 ACCAGCGCAC TTCGGCAGCG GCAGCACCTC GGCAGCGTCA GTGAAAATGC 50
circopormeeh 1 ACCAGCGCAC TTCGGCAGCG GCAGCACCTC GGCAGCGTCA GTGAAAATGC 50
circopordfp 1 ACCAGCGCAC TTCGGCAGCG GCAGCACCTC GGCAGCGTCA GTGAAAATGC 50
60 70 80 90 100
circopormank 51 CAAGCAAGAA AAGCGGCCCG CAACCCCATA AGAGGTGGGT GTTCACCCTT 100
circopormeeh 51 CAAGCAAGAA AAGCGGCCCG CAACCCCATA AGAGGTGGGT GTTCACCCTT 100
circopordfp 51 CAAGCAAGAA AAGCGGCCCG CAACCCCATA AGAGGTGGGT GTTCACCCTT 100
110 120 130 140 150
circopormank 101 AATAATCCTT CCGAGGAGGA GAAAAACAAA ATACGGGAGC TTCCAATCTC 150
circopormeeh 101 AATAATCCTT CCGAGGAGGA GAAAAACAAA ATACGGGAGC TTCCAATCTC 150
circopordfp 101 AATAATCCTT CCGAGGAGGA GAAAAACAAA ATACGGGAGC TTCCAATCTC 150
160 170 180 190 200
circopormank 151 CTTTTTGAT TATTTTGTTT GCGGAGAGGA AGGTTTGGAA GAGGGTAGAA 200
circopormeeh 151 CTTTTTGAT TATTTTGTTT GCGGAGAGGA AGGTTTGGAA GAGGGTAGAA 200
circopordfp 151 CTTTTTGAT TATTTTGTTT GTGGCGAGGA AGGTTTGGAA GAGGGTAGAA 200
210 220 230 240 250
circopormank 201 CTGCTCACCT CCAGGGGTTT GCTAATTTTG CTAAGAAGCA GACTTTTAAC 250
circopormeeh 201 CTCCTCACCT CCAGGGGTTT GCGAATTTTG CTAAGAAGCA GACTTTTAAC 250
circopordfp 201 CTCCTCACCT CCAGGGGTTT GCGAATTTTG CTAAGAAGCA GACTTTTAAC 250
260 270 280 290 300
circopormank 251 AAGGTGAAGT GGTATTTTGG TGCCCGCTGC CACATCGAGA AAGCGAAAGG 300
circopormeeh 251 AAGGTGAAGT GGTATTTTGG TGCCCGCTGC CACATCGAGA AAGCGAAAGG 300
circopordfp 251 AAGGTGAAGT GGTATTTTGG TGCCCGCTGC CACATCGAGA AAGCGAAAGG 300
310 320 330 340 350
circopormank 301 ACCGACCAG CAGAATAAAG AATACTGCAG TAAAGAAGGC CACATACTTA 350
circopormeeh 301 ACCGACCAG CAGAATAAAG AATACTGCAG TAAAGAAGGC CACATACTTA 350
circopordfp 301 ACCGACCAG CAGAATAAAG AATACTGCAG TAAAGAAGGC CACATACTTA 350
360 370 380 390 400
circopormank 351 TCGAGTGTGG AGCTCCGCGG AACCAGGGGA AGCGCAGCGA CCTGTCTACT 400
circopormeeh 351 TCGAGTGTGG AGCTCCGCGG AACCAGGGGA AGCGCAGCGA CCTGTCTACT 400
circopordfp 351 TCGAGTGTGG AGCTCCGCGG AACCAGGGGA AGCGCAGCGA CCTGTCTACT 400
410 420 430 440 450
circopormank 401 GCTGTGAGTA CCCTTTTGGA GACGGGGTCT TTGGTGACTG TAGCCGAGCA 450
circopormeeh 401 GCTGTGAGTA CCCTTTTGGA GACGGGGTCT TTGGTGACTG TAGCCGAGCA 450
circopordfp 401 GCTGTGAGTA CCCTTTTGGA GACGGGGTCT TTGGTGACTG TAGCCGAGCA 450
460 470 480 490 500
circopormank 451 GTTCCCTGTA ACGTATGTGA GAAATTTCCG CGGGCTGGCT GAACTTTTGA 500
circopormeeh 451 GTTCCCTGTA ACGTATGTGA GAAATTTCCG CGGGCTGGCT GAACTTTTGA 500
circopordfp 451 GTTTCCTGTA ACGTATGTGA GAAATTTCCG CGGGCTGGCT GAACTTTTGA 500
510 520 530 540 550
circopormank 501 AAGTGAGCGG GAAGATGCAG CAGCGTGATT GGAAGACAGC TGTACACGTC 550
circopormeeh 501 AAGTGAGCGG GAAGATGCAG CAGCGTGATT GGAAGACAGC TGTACACGTC 550
circopordfp 501 AAGTGAGCGG GAAGATGCAG CAGCGTGATT GGAAGACAGC TGTACACGTC 550
560 570 580 590 600
circopormank 551 ATAGTGGGCC CGCCCGGTTG TGGGAAGAGC CAGTGGGCCC GTAATTTTGC 600
circopormeeh 551 ATAGTGGGCC CGCCCGGTTG TGGGAAGAGC CAGTGGGCCC GTAATTTTGC 600
circopordfp 551 ATAGTGGGCC CGCCCGGTTG TGGGAAGAGC CAGTGGGCCC GTAATTTTGC 600

FIGURE 3

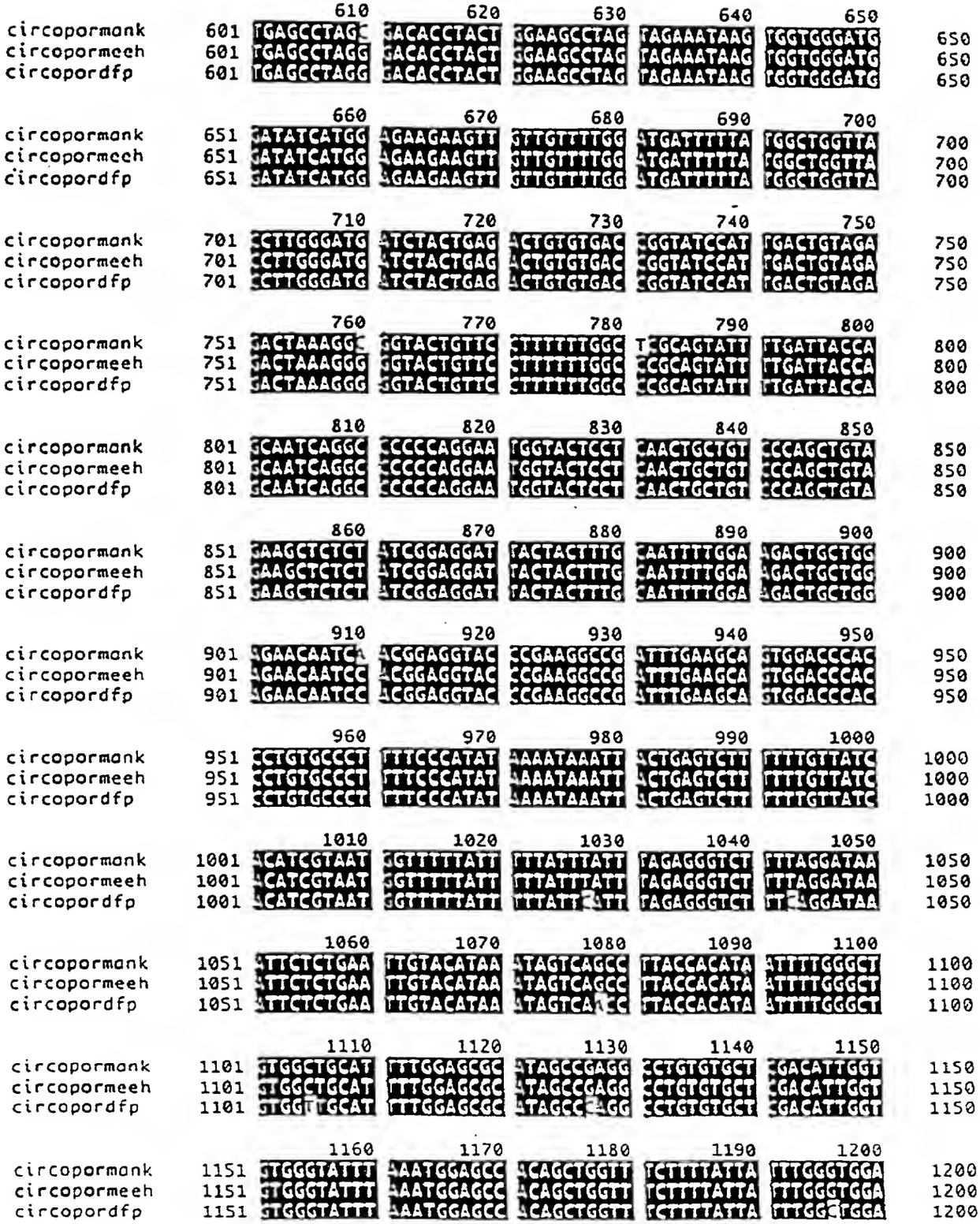


FIGURE 3 (continuation 1)

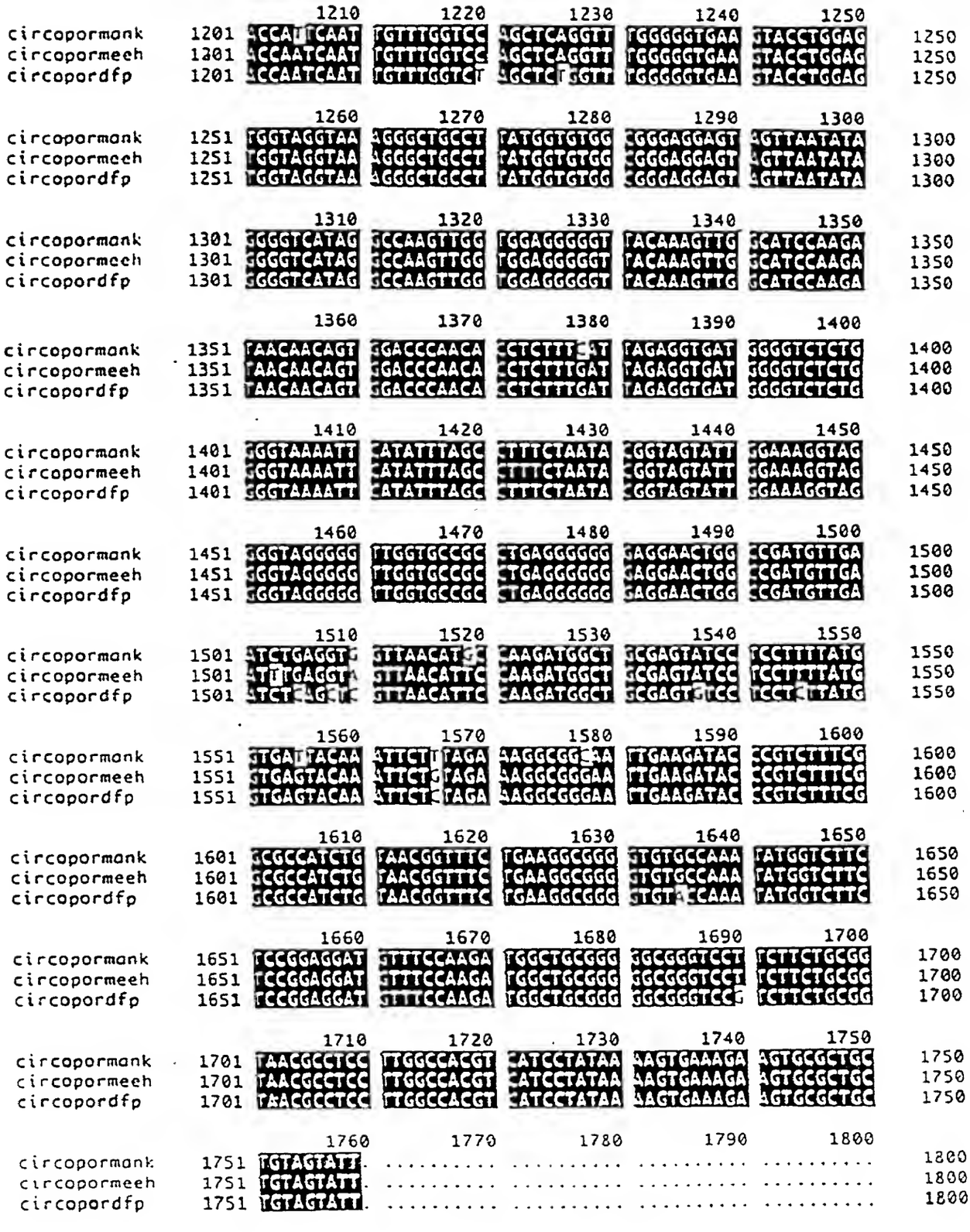


FIGURE 3 (continuation 2)

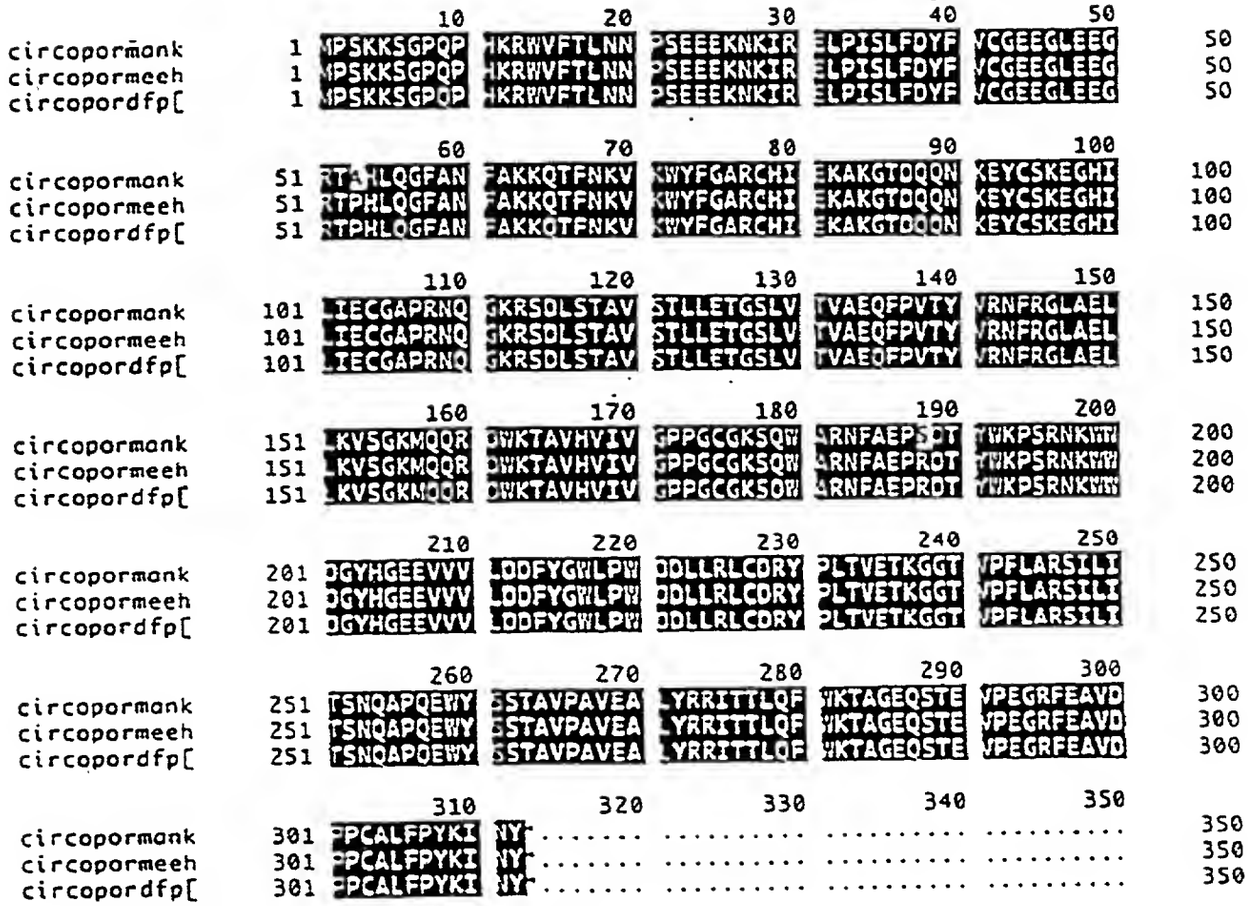

10 20 30 40 50
circopormank 1 MPSKKSGPQP HKRWVFTLNN PSEEEKNKIR ELPISLFDYF VCGEEGLEEG 50
circopormeeh 1 MPSKKSGPQP HKRWVFTLNN PSEEEKNKIR ELPISLFDYF VCGEEGLEEG 50
circopordfp[1 MPSKKSGPQP HKRWVFTLNN PSEEEKNKIR ELPISLFDYF VCGEEGLEEG 50
60 70 80 90 100
circopormank 51 RTAHLQGFAN FAKKQTFNKV KWYFGARCHI EKAKGTQQQN KEYCSKEGHI 100
circopormeeh 51 RTPHLQGFAN FAKKQTFNKV KWYFGARCHI EKAKGTQQQN KEYCSKEGHI 100
circopordfp[51 RTPHLQGFAN FAKKQTFNKV KWYFGARCHI EKAKGTQQQN KEYCSKEGHI 100
110 120 130 140 150
circopormank 101 LIECGAPRNQ GKRSDLSTAV STLLETGSLV TVAEQFPVTY VRNFRGLAEL 150
circopormeeh 101 LIECGAPRNQ GKRSDLSTAV STLLETGSLV TVAEQFPVTY VRNFRGLAEL 150
circopordfp[101 LIECGAPRNQ GKRSDLSTAV STLLETGSLV TVAEQFPVTY VRNFRGLAEL 150
160 170 180 190 200
circopormank 151 LKVSGKMQQR QWKTAVHVIV GPPGCGKSQW ARNFAEPSQT TWKPSRNKWW 200
circopormeeh 151 LKVSGKMQQR QWKTAVHVIV GPPGCGKSQW ARNFAEPROT TWKPSRNKWW 200
circopordfp[151 LKVSGKMQQR QWKTAVHVIV GPPGCGKSQW ARNFAEPROT TWKPSRNKWW 200
210 220 230 240 250
circopormank 201 QGYHGEEVVV LQDFYGWLPW QDLLRLCQRY PLTVETKGGT VPFLARSILI 250
circopormeeh 201 QGYHGEEVVV LQDFYGWLPW QDLLRLCQRY PLTVETKGGT VPFLARSILI 250
circopordfp[201 QGYHGEEVVV LQDFYGWLPW QDLLRLCQRY PLTVETKGGT VPFLARSILI 250
260 270 280 290 300
circopormank 251 TSNQAPQEWY SSTAVPAVEA LYRRITTLQF WKTAGEQSTE VPEGRFEAVD 300
circopormeeh 251 TSNQAPQEWY SSTAVPAVEA LYRRITTLQF WKTAGEQSTE VPEGRFEAVD 300
circopordfp[251 TSNQAPQEWY SSTAVPAVEA LYRRITTLQF WKTAGEQSTE VPEGRFEAVD 300
310 320 330 340 350
circopormank 301 PPCALFPYKI NY..... 350
circopormeeh 301 PPCALFPYKI NY..... 350
circopordfp[301 PPCALFPYKI NY..... 350

FIGURE 4

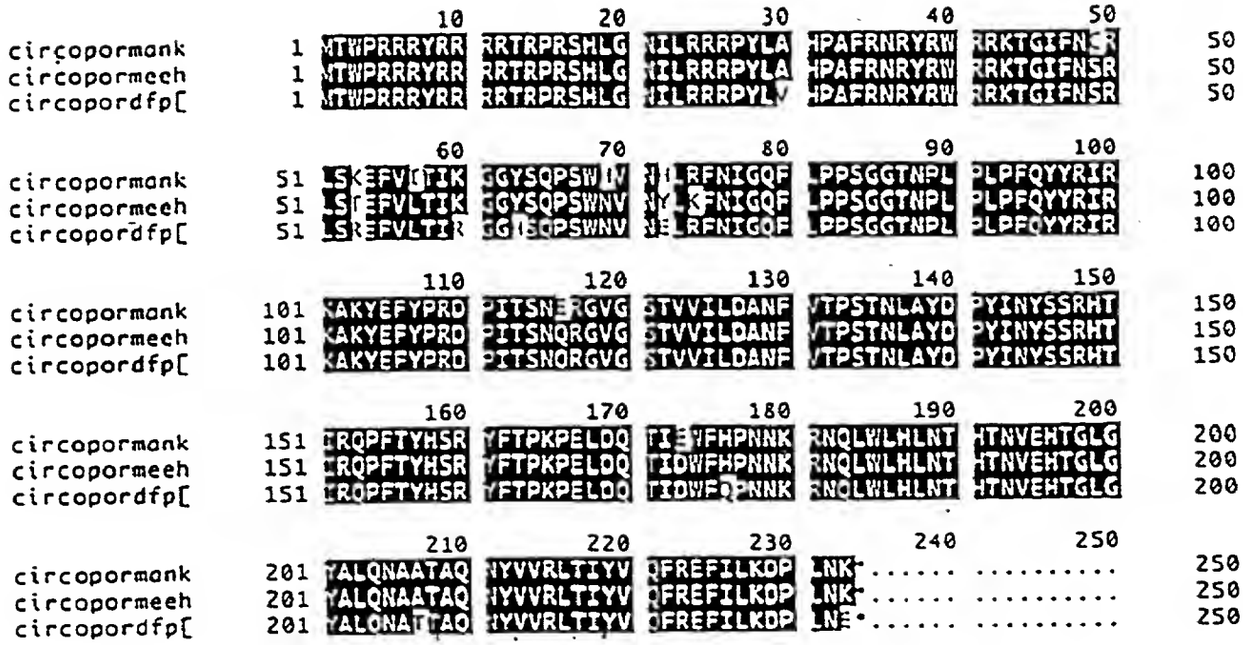


FIGURE 5

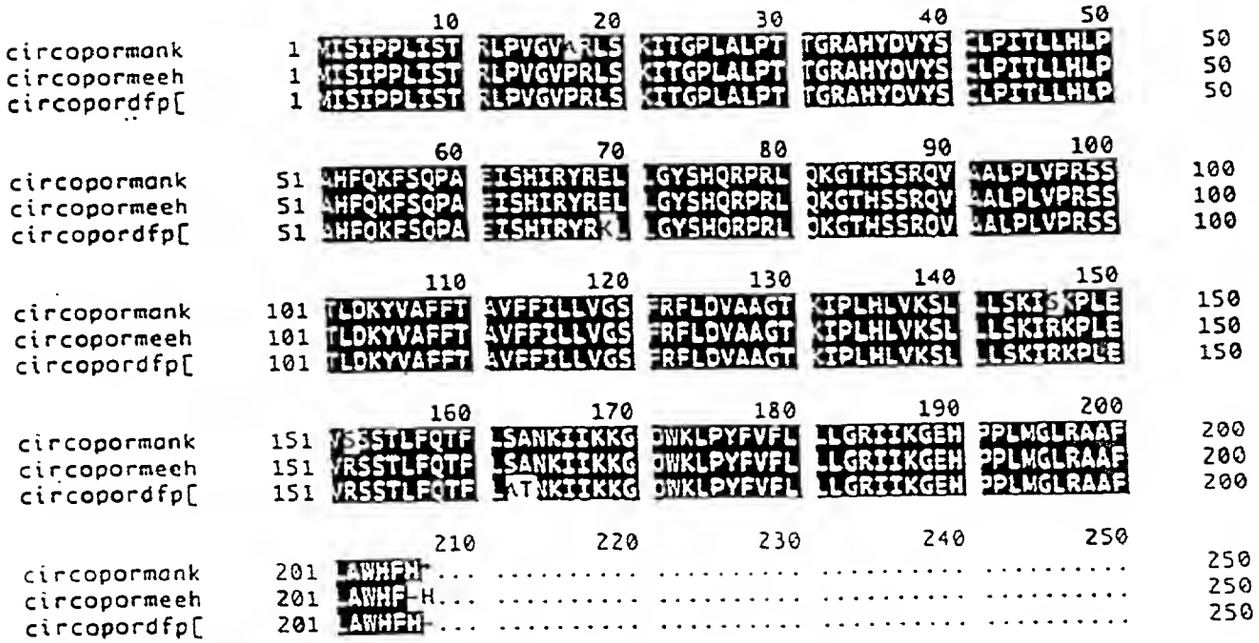


FIGURE 6

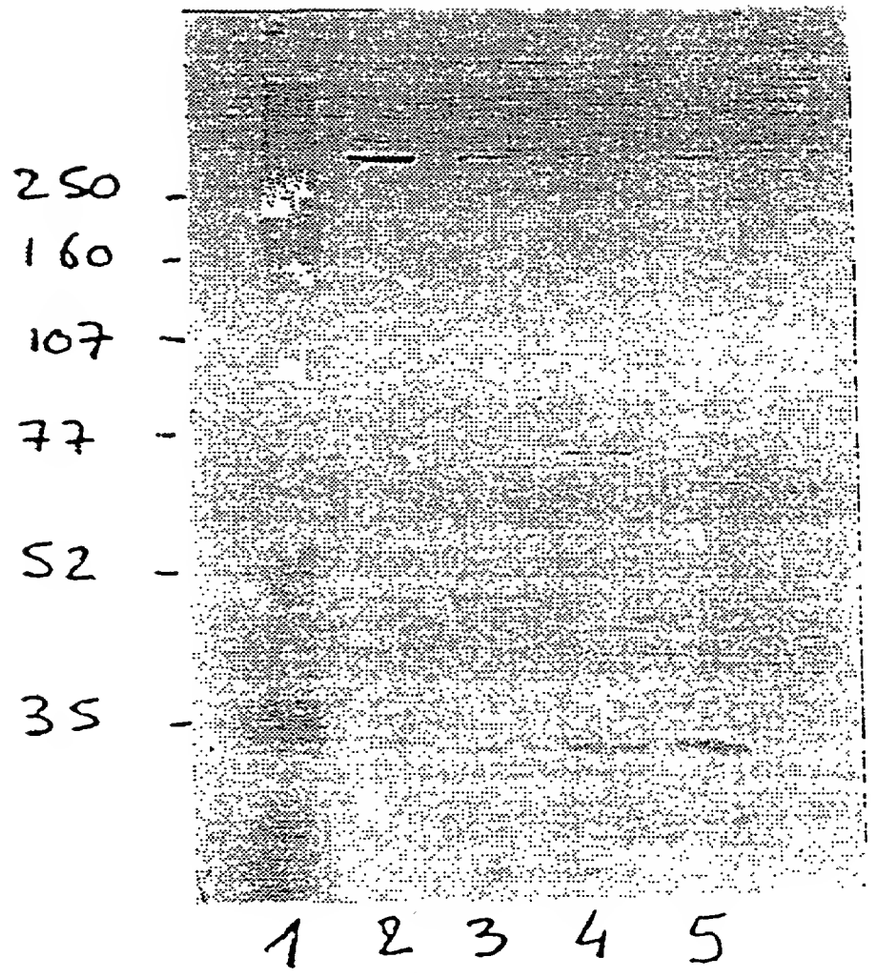


FIGURE 7

REPLACEMENT SHEET (RULE 26)

```
  Leu Ala Ser Arg Cys Arg Cys Cys Arg Pro Leu Val Glu Ala Ala Val His Gly
 Trp Arg Val Glu Ala Ala Ala Ala Gly Arg Cys Cys Arg Leu Leu Leu Met Gly
Gly Ala Cys Lys Pro Leu Pro Leu Val Glu Ala Ala Gly *** Cys Cys Cys Ala
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
3'  TGG TCG CGT GAA GCC GTC GCC GTC GTG GAG CCG TCG TGG AGT CGT CGT TGT ACG
              9          18          27          36          45          54
5'  ACC AGC GCA CTT CGG CAG CGG CAG CAC CTC GGC AGC ACC TCA GCA GCA ACA TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Ser Ala Leu Arg Gln Arg Gln His Leu Gly Ser Thr Ser Ala Ala Thr Cys
 Pro Ala His Phe Gly Ser Gly Ser Thr Ser Ala Ala Pro Gln Gln Gln His Ala
  Gln Arg Thr Ser Ala Ala Ala Ala Pro Arg Gln His Leu Ser Ser Asn Met Pro

  Ala Leu Leu Ile Ser Ser Ala Ser Gly Leu Gly Met Phe Pro Pro His Glu Ser
 Leu Leu Phe Phe Pro Leu Leu Pro Gly Trp Gly Trp Leu Leu His Thr Asn Val
Trp Cys Ser Ser His Phe Phe Arg Val Gly Val Gly Tyr Phe Thr Pro Thr ***
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GGT CGT TCT TCT TAC CTT CTT CGC CTG GGG TTG GGG TAT TTT CCA CCC ACA AGT
          63          72          81          90          99         108
CCA GCA AGA AGA ATG GAA GAA GCG GAC CCC AAC CCC ATA AAA GGT GGG TGT TCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Ala Arg Arg Met Glu Glu Ala Asp Pro Asn Pro Ile Lys Gly Gly Cys Ser
 Gln Gln Glu Glu Trp Lys Lys Arg Thr Pro Thr Pro *** Lys Val Gly Val His
  Ser Lys Lys Asn Gly Arg Ser Gly Pro Gln Pro His Lys Arg Trp Val Phe Thr

  Gln Ile Ile Arg Gly Phe Val Leu Ala Leu Phe Tyr Pro Ile Lys Trp Tyr Gly
 Arg Phe Leu Gly Glu Ser Ser Ser Arg Leu Phe Ile Arg Ser Arg Gly Ile Asp
Glu Ser Tyr Asp Lys Arg Leu Arg Ala Cys Ser Phe Val Pro Asp Glu Leu Ile
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GAG ACT TAT TAG GAA GGC TTC TGC TCG CGT TCT TTT ATG CCC TAG AAG GTT ATA
        117         126         135         144         153         162
CTC TGA ATA ATC CTT CCG AAG ACG AGC GCA AGA AAA TAC GGG ATC TTC CAA TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu *** Ile Ile Leu Pro Lys Thr Ser Ala Arg Lys Tyr Gly Ile Phe Gln Tyr
 Ser Glu *** Ser Phe Arg Arg Arg Ala Gln Glu Asn Thr Gly Ser Ser Asn Ile
  Leu Asn Asn Pro Ser Glu Asp Glu Arg Lys Lys Ile Arg Asp Leu Pro Ile Ser

  *** Lys Ile Ile Lys Asn Asn Ala Leu Leu Thr Ile Leu Phe Ser Ser Cys Arg
 Arg Asn Ser *** Lys Ile Thr Pro Ser Ser Pro Leu Ser Ser Pro Arg Val Gly
Gly Ile Gln Asn Asn *** Gln Gln Arg Pro Pro Tyr His Pro Leu Val Phe Val
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GGG ATA AAC TAA TAA AAT AAC AAC CGC TCC TCC CAT TAC TCC TTC CTG CTT GTG
        171         180         189         198         207         216
CCC TAT TTG ATT ATT TTA TTG TTG GCG AGG AGG GTA ATG AGG AAG GAC GAA CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Tyr Leu Ile Ile Leu Leu Leu Ala Arg Arg Val Met Arg Lys Asp Glu His
 Pro Ile *** Leu Phe Tyr Cys Trp Arg Gly Gly *** *** Gly Arg Thr Asn Thr
  Leu Phe Asp Tyr Phe Ile Val Gly Glu Glu Gly Asn Glu Glu Gly Arg Thr Pro

  Val Glu Leu Pro Glu Ser Ile Lys His Leu Leu Leu Ser Lys Ile Phe His Leu
 *** Arg Trp Pro Asn Ala Leu Lys Thr Phe Phe Cys Val Lys Leu Leu Thr Phe
Glu Gly Gly Pro Thr Arg *** Asn Gln Ser Ser Ala Ser Lys *** Tyr Leu Ser
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GAG TGG AGG TCC CCA AGC GAT TAA AAC ACT TCT TCG TCT GAA AAT TAT TTC ACT
        225         234         243         252         261         270
CTC ACC TCC AGG GGT TCG CTA ATT TTG TGA AGA AGC AGA CTT TTA ATA AAG TGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Thr Ser Arg Gly Ser Leu Ile Leu *** Arg Ser Arg Leu Leu Ile Lys ***
 Ser Pro Pro Gly Val Arg *** Phe Cys Glu Glu Ala Asp Phe *** *** Ser Glu
  His Leu Gln Gly Phe Ala Asn Phe Val Lys Lys Gln Thr Phe Asn Lys Val Lys
```

FIGURE 8

```
  Pro Ile Gln Thr Gly Ala Ala Val Asp Leu Phe Arg Phe Ser Cys Ile Leu Leu
 His Tyr Lys Pro Ala Arg Gln Trp Met Ser Phe Ala Phe Pro Val Ser *** Cys
Thr Thr Asn Pro His Gly Ser Gly Cys Arg Ser Leu Ser Leu Phe Leu Asp Ala
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TCA CCA TAA ACC CAC GGG CGA CGG TGT AGC TCT TTC GCT TTC CTT GTC TAG TCG
       279         288         297         306         315         324
AGT GGT ATT TGG GTG CCC GCT GCC ACA TCG AGA AAG CGA AAG GAA CAG ATC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Gly Ile Trp Val Pro Ala Ala Thr Ser Arg Lys Arg Lys Glu Gln Ile Ser
 Val Val Phe Gly Cys Pro Leu Pro His Arg Glu Ser Glu Arg Asn Arg Ser Ala
  Trp Tyr Leu Gly Ala Arg Cys His Ile Glu Lys Ala Lys Gly Thr Asp Gln Gln

  Ile Phe Phe Val Ala Thr Phe Phe Ala Val *** Gln His Leu Thr Ser Ser Arg
 Phe Leu Ser Tyr Gln Leu Leu Ser Pro Leu Lys Ser Ile Ser His Pro Ala Gly
Ser Tyr Leu Ile Ser Cys Tyr Leu Leu Cys Ser Val Ser Pro Thr His Leu Glu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TCT TAT TTC TTA TGA CGT CAT TTC TTC CGT TGA ATG ACT ACC TCA CAC CTC GAG
       333         342         351         360         369         378
AGA ATA AAG AAT ACT GCA GTA AAG AAG GCA ACT TAC TGA TGG AGT GTG GAG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Ile Lys Asn Thr Ala Val Lys Lys Ala Thr Tyr *** Trp Ser Val Glu Leu
 Glu *** Arg Ile Leu Gln *** Arg Arg Gln Leu Thr Asp Gly Val Trp Ser Ser
  Asn Lys Glu Tyr Cys Ser Lys Glu Gly Asn Leu Leu Met Glu Cys Gly Ala Pro

  Ser Arg Leu Ser Leu Pro Thr Val Gln Arg Ser Ser His Thr Gly Gln Gln Leu
 Leu Asp *** Pro Cys Arg Leu Ser Arg Asp Val Ala Thr Leu Val Lys Asn Ser
*** Ile Glu Pro Val Val Ser His Gly Thr *** Gln Gln Ser Tyr Arg Thr Pro
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GAT CTA GAG TCC CTG TTG CCT CAC TGG ACA GAT GAC GAC ACT CAT GGA ACA ACC
       387         396         405         414         423         432
CTA GAT CTC AGG GAC AAC GGA GTG ACC TGT CTA CTG CTG TGA GTA CCT TGT TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Asp Leu Arg Asp Asn Gly Val Thr Cys Leu Leu Leu *** Val Pro Cys Trp
 *** Ile Ser Gly Thr Thr Glu *** Pro Val Tyr Cys Cys Glu Tyr Leu Val Gly
  Arg Ser Gln Gly Gln Arg Ser Asp Leu Ser Thr Ala Val Ser Thr Leu Leu Glu

  Ala Pro Thr Gln His Gly Asn Cys Leu Leu Val Arg Tyr Arg Lys Asp Ser Ile
 Leu Pro Leu Arg Thr Val Thr Ala Ser Cys Cys Gly Thr Val Asn Thr Leu Phe
Ser Arg Ser Asp Pro Ser Arg Gln Leu Ala Ala Gly Gln Leu Thr Gln *** Phe
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TCT CGC CCT CAG ACC ACT GGC AAC GTC TCG TCG TGG GAC ATT GCA AAC AGT CTT
       441         450         459         468         477         486
AGA GCG GGA GTC TGG TGA CCG TTG CAG AGC AGC ACC CTG TAA CGT TTG TCA GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Ala Gly Val Trp *** Pro Leu Gln Ser Ser Thr Leu *** Arg Leu Ser Glu
 Glu Arg Glu Ser Gly Asp Arg Cys Arg Ala Ala Pro Cys Asn Val Cys Gln Lys
  Ser Gly Ser Leu Val Thr Val Ala Glu Gln His Pro Val Thr Phe Val Arg Asn

  Glu Ala Pro Gln Ser Phe Lys Gln Phe His Ala Pro Phe His Leu Leu Thr Ile
 Lys Arg Pro Ser Ala Ser Ser Lys Phe Thr Leu Pro Phe Ile Cys Phe Arg Ser
Asn Gly Arg Ala Pro Gln Val Lys Ser Leu Ser Arg Ser Phe Ala Ser Ala His
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TAA AGG CGC CCG ACC GAC TTG AAA ACT TTC ACT CGC CCT TTT ACG TCT TCG CAC
       495         504         513         522         531         540
ATT TCC GCG GGC TGG CTG AAC TTT TGA AAG TGA GCG GGA AAA TGC AGA AGC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Ser Ala Gly Trp Leu Asn Phe *** Lys *** Ala Gly Lys Cys Arg Ser Val
 Phe Pro Arg Ala Gly *** Thr Phe Glu Ser Glu Arg Glu Asn Ala Glu Ala ***
  Phe Arg Gly Leu Ala Glu Leu Leu Lys Val Ser Gly Lys Met Gln Lys Arg Asp
```

FIGURE 8 (continuation 1)

```
  Pro Leu Ser Ile Tyr Val Asp Asn His Pro Trp Arg Pro Thr Thr Phe Ala Phe
 Gln Phe Val Leu Thr Cys Thr Met Thr Pro Gly Gly Pro His Pro Leu Leu Leu
Asn Ser Ser *** His Val Arg *** Gln Pro Ala Val Gln Thr His Tyr Phe Cys
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TAA CCT TCT GAT TAC ATG TGC AGT AAC ACC CCG GTG GAC CCA CAC CAT TTT CGT
        549         558         567         576         585         594
ATT GGA AGA CTA ATG TAC ACG TCA TTG TGG GGC CAC CTG GGT GTG GTA AAA GCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Gly Arg Leu Met Tyr Thr Ser Leu Trp Gly His Leu Gly Val Val Lys Ala
 Leu Glu Asp *** Cys Thr Arg His Cys Gly Ala Thr Trp Val Trp *** Lys Gln
  Trp Lys Thr Asn Val His Val Ile Val Gly Pro Pro Gly Cys Gly Lys Ser Lys

  Pro Ser Ser Ile Lys Cys Val Arg Phe Gly Cys Val Pro Phe Trp Arg Ser Val
 His Ala Ala Leu Lys Ala Ser Gly Ser Val Val Tyr Gln Phe Gly Gly Leu Phe
Ile Pro Gln *** Asn Gln Leu Gly Pro Phe Trp Met Ser Ser Val Val *** Phe
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TTA CCC GAC GAT TAA AAC GTC TGG GCC TTT GGT GTA TGA CCT TTG GTG GAT CTT
        603         612         621         630         639         648
AAT GGG CTG CTA ATT TTG CAG ACC CGG AAA CCA CAT ACT GGA AAC CAC CTA GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Gly Leu Leu Ile Leu Gln Thr Arg Lys Pro His Thr Gly Asn His Leu Glu
 Met Gly Cys *** Phe Cys Arg Pro Gly Asn His Ile Leu Glu Thr Thr *** Lys
  Trp Ala Ala Asn Phe Ala Asp Pro Glu Thr Thr Tyr Trp Lys Pro Pro Arg Asn

  Leu Pro Pro Ile Thr Val Met Thr Phe Phe His Asn Asn Asn Ile Val Lys Ile
 Leu His His Ser Pro *** Trp Pro Ser Ser Thr Thr Thr Ile Ser Ser Lys ***
Cys Thr Thr Pro His Asn Gly His His Leu Leu Pro Gln *** Gln His Ser Lys
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TGT TCA CCA CCC TAC CAA TGG TAC CAC TTC TTC ACC AAC AAT AAC TAC TGA AAA
        657         666         675         684         693         702
ACA AGT GGT GGG ATG GTT ACC ATG GTG AAG AAG TGG TTG TTA TTG ATG ACT TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Ser Gly Gly Met Val Thr Met Val Lys Lys Trp Leu Leu Leu Met Thr Phe
 Gln Val Val Gly Trp Leu Pro Trp *** Arg Ser Gly Cys Tyr *** *** Leu Leu
  Lys Trp Trp Asp Gly Tyr His Gly Glu Glu Val Val Val Ile Asp Asp Phe Tyr

  Ala Pro Gln Gly Pro Ile Ile *** Gln Ser Gln Thr Ile Ser Ile Trp Gln Ser
 Pro Gln Ser Gly Gln Ser Ser Arg Ser Leu Ser His Ser Arg Tyr Gly Asn Val
His Ser Ala Ala Arg Pro His Asp Val Ser Val Thr His Asp Ile Asp Met Ser
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TAC CGA CCG ACG GGA CCC TAC TAG ATG ACT CTG ACA CAC TAG CTA TAG GTA ACT
        711         720         729         738         747         756
ATG GCT GGC TGC CCT GGG ATG ATC TAC TGA GAC TGT GTG ATC GAT ATC CAT TGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Met Ala Gly Cys Pro Gly Met Ile Tyr *** Asp Cys Val Ile Asp Ile His ***
 Trp Leu Ala Ala Leu Gly *** Ser Thr Glu Thr Val *** Ser Ile Ser Ile Asp
  Gly Trp Leu Pro Trp Asp Asp Leu Leu Arg Leu Cys Asp Arg Tyr Pro Leu Thr

  Tyr Leu Ser Phe Thr Ser Ser Tyr Arg Lys Gln Gly Ala Thr Asn Gln Asn Gly
 Thr Ser Val Leu Pro Pro Val Thr Gly Lys Lys Ala Arg Leu Ile Arg Ile Val
Gln Leu Ser *** Leu His Phe Gln Val Lys Lys Pro Gly Cys Tyr Glu Ser ***
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GAC ATC TCT GAT TTC CAC CTT GAC ATG GAA AAA ACC GGG CGT CAT AAG ACT AAT
        765         774         783         792         801         810
CTG TAG AGA CTA AAG GTG GAA CTG TAC CTT TTT TGG CCC GCA GTA TTC TGA TTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu *** Arg Leu Lys Val Glu Leu Tyr Leu Phe Trp Pro Ala Val Phe *** Leu
 Cys Arg Asp *** Arg Trp Asn Cys Thr Phe Phe Gly Pro Gln Tyr Ser Asp Tyr
  Val Glu Thr Lys Gly Gly Thr Val Pro Phe Leu Ala Arg Ser Ile Leu Ile Thr
```

FIGURE 8 (continuation 2)

```
  Ala Ile Leu Gly Arg Gln Phe Pro Val Gly *** Ser Ser Asp Trp Ser Tyr Phe
 Leu Leu *** Val Gly Asn Ser His Tyr Glu Glu Val Ala Thr Gly Ala Thr Ser
Trp Cys Asp Ser Gly Thr Pro Ile Thr Ser Arg Leu Gln Gln Gly Leu Gln Leu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GGT CGT TAG TCT GGG GCA ACC TTA CCA TGA GGA GTT GAC GAC AGG GTC GAC ATC
        819         828         837         846         855         864
CCA GCA ATC AGA CCC CGT TGG AAT GGT ACT CCT CAA CTG CTG TCC CAG CTG TAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Ala Ile Arg Pro Arg Trp Asn Gly Thr Pro Gln Leu Leu Ser Gln Leu ***
 Gln Gln Ser Asp Pro Val Gly Met Val Leu Leu Asn Cys Cys Pro Ser Cys Arg
  Ser Asn Gln Thr Pro Leu Glu Trp Tyr Ser Ser Thr Ala Val Pro Ala Val Glu

  Ser Lys Ile Pro Pro Asn Ser Gly Gln Tyr Lys Pro Leu Ile Ser Cys Phe Leu
 Ala Arg *** Arg Leu Ile Val Glu Lys Thr Asn Gln Phe Phe Ala Val Ser Cys
Leu Glu Lys Asp Ser Ser *** Lys Arg Pro Ile Lys Ser Ser His *** Leu Val
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TTC GAG AAA TAG CCT CCT AAT GAA GGA ACC ATA AAA CCT TCT TAC GAT GTC TTG
        873         882         891         900         909         918
AAG CTC TTT ATC GGA GGA TTA CTT CCT TGG TAT TTT GGA AGA ATG CTA CAG AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Leu Phe Ile Gly Gly Leu Leu Pro Trp Tyr Phe Gly Arg Met Leu Gln Asn
 Ser Ser Leu Ser Glu Asp Tyr Phe Leu Gly Ile Leu Glu Glu Cys Tyr Arg Thr
  Ala Leu Tyr Arg Arg Ile Thr Ser Leu Val Phe Trp Lys Asn Ala Thr Glu Gln

  Gly Arg Leu Phe Pro Ala Leu Glu Asp Gly Lys Gly Gly Trp Ala Arg Phe Lys
 Asp Val Ser Ser Pro Pro Trp Asn Thr Val Arg Glu Gly Gly His Gly Ser Asn
Ile Trp Pro Pro Leu Pro Gly Thr Arg *** Gly Lys Gly Gly Met Gly Gln Ile
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TTA GGT GCC TCC TTC CCC CGG TCA AGC AGT GGG AAA GGG GGG GTA CGG GAC TTA
        927         936         945         954         963         972
AAT CCA CGG AGG AAG GGG GCC AGT TCG TCA CCC TTT CCC CCC CAT GCC CTG AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Pro Arg Arg Lys Gly Ala Ser Ser Ser Pro Phe Pro Pro His Ala Leu Asn
 Ile His Gly Gly Arg Gly Pro Val Arg His Pro Phe Pro Pro Met Pro *** Ile
  Ser Thr Glu Glu Gly Gly Gln Phe Val Thr Leu Ser Pro Pro Cys Pro Glu Phe

  Trp Ile Phe Tyr Ile Val Ser Asp Lys Lys Asp Ser Arg Leu Pro Lys *** ***
 Gly Tyr Ser Ile Phe *** Gln Thr Lys Lys Ile Val Glu Tyr His Asn Lys Asn
Glu Met His Phe Leu Asn Ser Leu Arg Lys *** *** Lys Thr Ile Thr Lys Ile
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAG GTA TAC TTT ATT TAA TGA CTC AGA AAA AAT AGT GAA GCA TTA CCA AAA ATA
        981         990         999        1008        1017        1026
TTC CAT ATG AAA TAA ATT ACT GAG TCT TTT TTA TCA CTT CGT AAT GGT TTT TAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe His Met Lys *** Ile Thr Glu Ser Phe Leu Ser Leu Arg Asn Gly Phe Tyr
 Ser Ile *** Asn Lys Leu Leu Ser Leu Phe Tyr His Phe Val Met Val Phe Ile
  Pro Tyr Glu Ile Asn Tyr *** Val Phe Phe Ile Thr Ser *** Trp Phe Leu Leu

  Glu Asn Leu Thr Leu His Pro Thr Lys Leu Ile Leu Asn Glu Ser Asn Tyr Met
 Asn Met Leu Pro *** Thr Pro Pro Arg *** Phe *** Ile Arg Gln Ile Thr Cys
Ile *** *** Pro Asn Leu Pro Pro Asp Lys Phe Asn Phe Glu Arg Phe Gln Val
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ATA AGT AAT TCC CAA TTC ACC CCC CAG AAA TTT TAA TTT AAG AGA CTT AAC ATG
       1035        1044        1053        1062        1071        1080
TAT TCA TTA AGG GTT AAG TGG GGG GTC TTT AAA ATT AAA TTC TCT GAA TTG TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Ser Leu Arg Val Lys Trp Gly Val Phe Lys Ile Lys Phe Ser Glu Leu Tyr
 Ile His *** Gly Leu Ser Gly Gly Ser Leu Lys Leu Asn Ser Leu Asn Cys Thr
  Phe Ile Lys Gly *** Val Gly Gly Leu *** Asn *** Ile Leu *** Ile Val His
```

FIGURE 8 (continuation 3)

```
  Cys Pro *** Val Ser Ile Thr Asn Arg Thr Thr Tyr Val Thr Lys Ser Arg Leu
 Val His Asn Cys Pro Tyr Gln Ile Gly Pro Arg Ile Tyr Gln Lys Arg Val Cys
Tyr Met Thr Val Arg Ile Asn Tyr Glu Gln Asp Tyr Ile Ser Asn Glu Phe Ala
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TAT GTA CCA ATG TGC CTA TAA CAT AAG GAC CAG CAT ATA TGA CAA AAG CTT GCG
       1089        1098        1107        1116        1125        1134
ATA CAT GGT TAC ACG GAT ATT GTA TTC CTG GTC GTA TAT ACT GTT TTC GAA CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile His Gly Tyr Thr Asp Ile Val Phe Leu Val Val Tyr Thr Val Phe Glu Arg
 Tyr Met Val Thr Arg Ile Leu Tyr Ser Trp Ser Tyr Ile Leu Phe Ser Asn Ala
  Thr Trp Leu His Gly Tyr Cys Ile Pro Gly Arg Ile Tyr Cys Phe Arg Thr Gln

  Ala Ser Ala *** Thr Thr *** Met Glu Leu Leu Lys Tyr Asp *** Gly Cys Ser
 His Arg Pro Arg Arg Pro Arg Cys Lys Trp Cys Asn Thr Thr Glu Ala Val Ala
Thr Gly Leu Gly Val His Asp Val Asn Gly Ala Thr Gln Leu Arg Leu Trp Leu
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TCA CGG CTC CGG ATG CAC CAG ATG TAA AGG TCG TCA AAC ATC AGA GTC GGT GTC
       1143        1152        1161        1170        1179        1188
AGT GCC GAG GCC TAC GTG GTC TAC ATT TCC AGC AGT TTG TAG TCT CAG CCA CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Ala Glu Ala Tyr Val Val Tyr Ile Ser Ser Ser Leu *** Ser Gln Pro Gln
 Val Pro Arg Pro Thr Trp Ser Thr Phe Pro Ala Val Cys Ser Leu Ser His Ser
  Cys Arg Gly Leu Arg Gly Leu His Phe Gln Gln Phe Val Val Ser Ala Thr Ala

  Thr Glu Lys Thr Thr Gln Asn Ser Thr Ile Leu Leu Ser Ile *** Ser Leu Asn
 Pro Lys Lys Gln Gln Lys Thr Pro Leu Leu *** Tyr His Phe Arg Pro Cys Thr
Gln Asn Arg Lys Asn Asn Pro Gln Phe Tyr Asp Ile Thr Phe Asp Leu Val Pro
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GAC CAA AGA AAA CAA CAA ACC AAC CTT CAT TAG TTA TCA CTT TAG ATC CTG TCC
       1197        1206        1215        1224        1233        1242
CTG GTT TCT TTT GTT GTT TGG TTG GAA GTA ATC AAT AGT GAA ATC TAG GAC AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Val Ser Phe Val Val Trp Leu Glu Val Ile Asn Ser Glu Ile *** Asp Arg
 Trp Phe Leu Leu Leu Phe Gly Trp Lys *** Ser Ile Val Lys Ser Arg Thr Gly
  Gly Phe Phe Cys Cys Leu Val Gly Ser Asn Gln *** *** Asn Leu Gly Gln Val

  Pro Pro Leu Thr Gly Pro Thr Thr Pro Ser Pro Ser Pro *** Pro Ile Ala Pro
 Gln Pro Tyr Leu Val Pro Leu Pro Leu Leu Leu Ala Pro Asn His Tyr Pro Pro
Lys Pro Thr Phe Tyr Arg Ser His Tyr Ser Phe Pro Gln Thr Ile Thr His Arg
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
AAA CCC CCA TTT CAT GGC CCT CAC CAT CCT CTT CCC GAC CCA ATA CCA TAC CGC
       1251        1260        1269        1278        1287        1296
TTT GGG GGT AAA GTA CCG GGA GTG GTA GGA GAA GGG CTG GGT TAT GGT ATG GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Gly Gly Lys Val Pro Gly Val Val Gly Glu Gly Leu Gly Tyr Gly Met Ala
 Leu Gly Val Lys Tyr Arg Glu Trp *** Glu Lys Gly Trp Val Met Val Trp Arg
  Trp Gly *** Ser Thr Gly Ser Gly Arg Arg Arg Ala Gly Leu Trp Tyr Gly Gly

  Pro Thr Thr *** Met Pro Thr Met Pro Ser Pro Gln Pro Arg Gln *** Leu Thr
 Leu Leu Leu Lys Cys Leu Pro *** Leu His Pro Ser His Gly Lys Asn Cys Leu
Ser Ser Tyr Asn Val Tyr Pro Asp Tyr Thr Leu Ala Thr Ala Lys Thr Val Phe
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CCT CCT CAT CAA ATG TAT CCC CAG TAT CCA CTC CCG ACA CCG GAA ACA ATG TTT
       1305        1314        1323        1332        1341        1350
GGA GGA GTA GTT TAC ATA GGG GTC ATA GGT GAG GGC TGT GGC CTT TGT TAC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Gly Val Val Tyr Ile Gly Val Ile Gly Glu Gly Cys Gly Leu Cys Tyr Lys
 Glu Glu *** Phe Thr *** Gly Ser *** Val Arg Ala Val Ala Phe Val Thr Lys
  Arg Ser Ser Leu His Arg Gly His Arg *** Gly Leu Trp Pro Leu Leu Gln Ser
```

FIGURE 8 (continuation 4)

```
  Ile Met *** Phe Leu Leu Val Pro Ala Trp Glu Gly Thr Val Arg Pro Ser Arg
 *** *** Arg Phe Tyr Cys Cys Gln Leu Gly Ser Gly Gln *** Gly Pro His Asp
Asn Asp Asp Leu Ile Val Ala Ser Ser Gly Val Gly Arg Asp Gly Gln Thr Ile
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CAA TAG TAG ATT TTA TTG TCG TGA CCT CGG GTG AGG GGA CAG TGG GAC CCA CTA
       1359        1368        1377        1386        1395        1404
GTT ATC ATC TAA AAT AAC AGC ACT GGA GCC CAC TCC CCT GTC ACC CTG GGT GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Ile Ile *** Asn Asn Ser Thr Gly Ala His Ser Pro Val Thr Leu Gly Asp
 Leu Ser Ser Lys Ile Thr Ala Leu Glu Pro Thr Pro Leu Ser Pro Trp Val Ile
  Tyr His Leu Lys *** Gln His Trp Ser Pro Leu Pro Cys His Pro Gly *** Ser

  Pro Ala Pro Gly Ser Asn Leu Arg Leu Arg Glu *** Glu Thr Thr Asn Leu Pro
 Pro Leu Leu Ala Leu Ile *** Gly *** Gly Lys Lys Asn Gln Leu Ile *** Leu
Pro Ser Cys Pro Trp Phe Glu Val Lys Val Lys Arg Ile Arg Tyr Tyr Glu Phe
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GCC CCT CGT CCC GGT CTT AAG TTG GAA TTG GAA AGA ATA AGA CAT CAT AAG TTT
       1413        1422        1431        1440        1449        1458
CGG GGA GCA GGG CCA GAA TTC AAC CTT AAC CTT TCT TAT TCT GTA GTA TTC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Gly Ala Gly Pro Glu Phe Asn Leu Asn Leu Ser Tyr Ser Val Val Phe Lys
 Gly Glu Gln Gly Gln Asn Ser Thr Leu Thr Phe Leu Ile Leu *** Tyr Ser Lys
  Gly Ser Arg Ala Arg Ile Gln Pro *** Pro Phe Leu Phe Cys Ser Ile Gln Arg

  Cys Leu Ala Pro Thr Gln Gly Gly Glu Gln Pro Phe Phe Thr Met Leu Ile Ser
 Ala Cys Leu Pro Pro Lys Val Gly Arg Arg Pro Ser Ser Leu *** *** Tyr Gln
Pro Val Ser Arg Pro Asn Ser Gly Gly Gly Pro Pro Leu Phe Asp Asn Ile Asn
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CCC GTG TCT CGC CCC CAA ACT GGG GGG AGG ACC CCC TTC TTT CAG TAA TTA TAA
       1467        1476        1485        1494        1503        1512
GGG CAC AGA GCG GGG GTT TGA CCC CCC TCC TGG GGG AAG AAA GTC ATT AAT ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly His Arg Ala Gly Val *** Pro Pro Ser Trp Gly Lys Lys Val Ile Asn Ile
 Gly Thr Glu Arg Gly Phe Asp Pro Pro Pro Gly Gly Arg Lys Ser Leu Ile Leu
  Ala Gln Ser Gly Gly Leu Thr Pro Leu Leu Gly Glu Glu Ser His *** Tyr ***

  Asp *** *** Thr Trp Arg Gly Pro Pro Arg Glu Ser Gln Pro Glu Ser Ser Leu
 Ile Glu Asp His Gly Gly Gly Leu Leu Ala Asn Gln Ser His Asn Ala Gln Cys
Phe Arg Met Met Asp Val Ala Trp Ser Pro Thr Arg Val Thr Thr Arg Lys Val
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CTT AGA GTA GTA CAG GTG GCG GGT CCT CCC GCA AGA CTG ACA CCA AGC GAA CTG
       1521        1530        1539        1548        1557        1566
GAA TCT CAT CAT GTC CAC CGC CCA GGA GGG CGT TCT GAC TGT GGT TCG CTT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Ser His His Val His Arg Pro Gly Gly Arg Ser Asp Cys Gly Ser Leu Asp
 Asn Leu Ile Met Ser Thr Ala Gln Glu Gly Val Leu Thr Val Val Arg Leu Thr
  Ile Ser Ser Cys Pro Pro Pro Arg Arg Ala Phe *** Leu Trp Phe Ala *** Gln

  Ile Asp Ser Pro Ala Pro Ser Ala Pro Thr Ser Ser Ala Met Lys Gly Glu Gly
 Tyr Ile Arg Leu His Pro Leu Pro Pro His Gln Leu His Trp Lys Glu Lys Glu
Thr Tyr Gly Phe Thr Arg Ser Leu Arg Thr Asn Phe Ile Gly Asn Lys Arg Arg
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
TCA TAT AGG CTT CCA CGC CCT CTC CGC CCA CAA CTT CTA CGG TAA AAA GGA AGA
       1575        1584        1593        1602        1611        1620
AGT ATA TCC GAA GGT GCG GGA GAG GCG GGT GTT GAA GAT GCC ATT TTT CCT TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Ile Ser Glu Gly Ala Gly Glu Ala Gly Val Glu Asp Ala Ile Phe Pro Ser
 Val Tyr Pro Lys Val Arg Glu Arg Arg Val Leu Lys Met Pro Phe Phe Leu Leu
  Tyr Ile Arg Arg Cys Gly Arg Gly Gly Cys *** Arg Cys His Phe Ser Phe Ser
```

FIGURE 8 (continuation 5)

```
  Ala Thr Val Thr Ala Pro Thr Ser Ser Gly Pro Ala Ala Ala Ser Ser Arg Ala
 Leu Pro Leu Pro Pro Pro Pro Pro Arg Ala Leu Pro Pro Pro Pro Pro Asp Pro
Trp Arg Tyr Arg His Arg Pro His Val Leu Trp Pro Arg Arg Arg Leu Ile Gln
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GGT CGC CAT TGC CAC CGC CCC CAC CTG CTC GGT CCC CGC CGC CGC CTC CTA GAC
       1629        1638        1647        1656        1665        1674
CCA GCG GTA ACG GTG GCG GGG GTG GAC GAG CCA GGG GCG GCG GCG GAG GAT CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Ala Val Thr Val Ala Gly Val Asp Glu Pro Gly Ala Ala Ala Glu Asp Leu
 Gln Arg *** Arg Trp Arg Gly Trp Thr Ser Gln Gly Arg Arg Arg Arg Ile Trp
  Ser Gly Asn Gly Gly Gly Gly Gly Arg Ala Arg Gly Gly Gly Gly Gly Ser Gly

  Leu Ile Ala Ala Pro Ala Thr Asp Glu Glu Glu Thr Val Gly Gly Gln Ile Arg
 Trp Ser Pro Gln Pro Pro Pro Thr Lys Lys Lys Pro Leu Ala Glu Lys Ser Val
Gly Leu His Ser Arg Pro Arg His Arg Arg Arg Arg Tyr Arg Arg Arg Pro Tyr
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
CGG TTC TAC CGA CGC CCC CGC CAC AGA AGA AGA AGC CAT TGC GGA GGA ACC TAT
       1683        1692        1701        1710        1719        1728
GCC AAG ATG GCT GCG GGG GCG GTG TCT TCT TCT TCG GTA ACG CCT CCT TGG ATA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Lys Met Ala Ala Gly Ala Val Ser Ser Ser Ser Val Thr Pro Pro Trp Ile
 Pro Arg Trp Leu Arg Gly Arg Cys Leu Leu Leu Arg *** Arg Leu Leu Gly Tyr
  Gln Asp Gly Cys Gly Gly Gly Val Phe Phe Phe Gly Asn Ala Ser Leu Asp Thr

  *** Ile Gln Phe Arg Phe Phe His Ala Thr Leu Ile
 Asp Tyr Arg Phe Val Phe Ser Thr Arg Gln Leu Tyr
Thr Met Asp Ser Phe Ser Leu Leu Ala Ser Tyr Thr Asn
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
GCA GTA TAG ACT TTT GCT TTC TTC ACG CGA CAT TCA TAA 5'
       1737        1746        1755        1764
CGT CAT ATC TGA AAA CGA AAG AAG TGC GCT GTA AGT ATT 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg His Ile *** Lys Arg Lys Lys Cys Ala Val Ser Ile
 Val Ile Ser Glu Asn Glu Arg Ser Ala Leu *** Val
  Ser Tyr Leu Lys Thr Lys Glu Val Arg Cys Lys Tyr
```

FIGURE 8 (continuation 6)

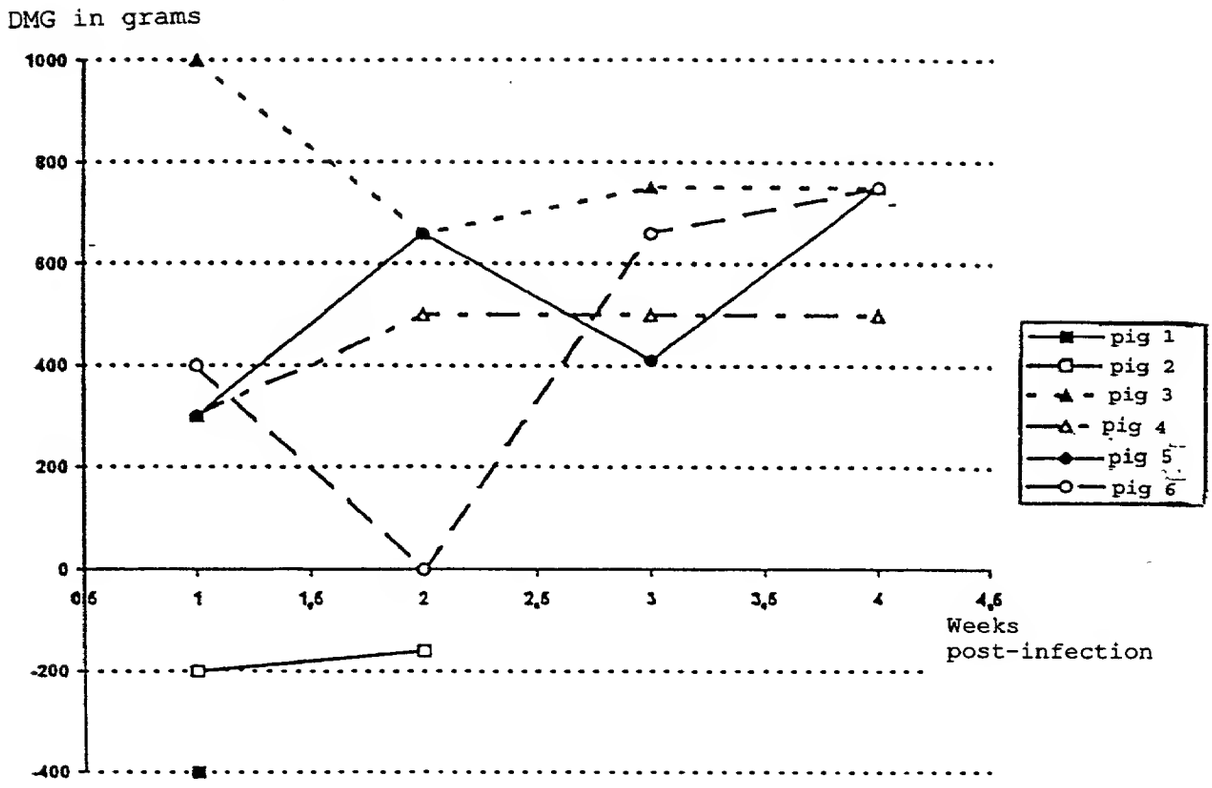

DMG in grams
1000
800
600
400
200
0
-200
-400
0,5
1
1,5
2
2,5
3
3,5
4
4,5
Weeks
post-infection
pig 1
pig 2
pig 3
pig 4
pig 5
pig 6

FIGURE 9

dmg: d0 - d28

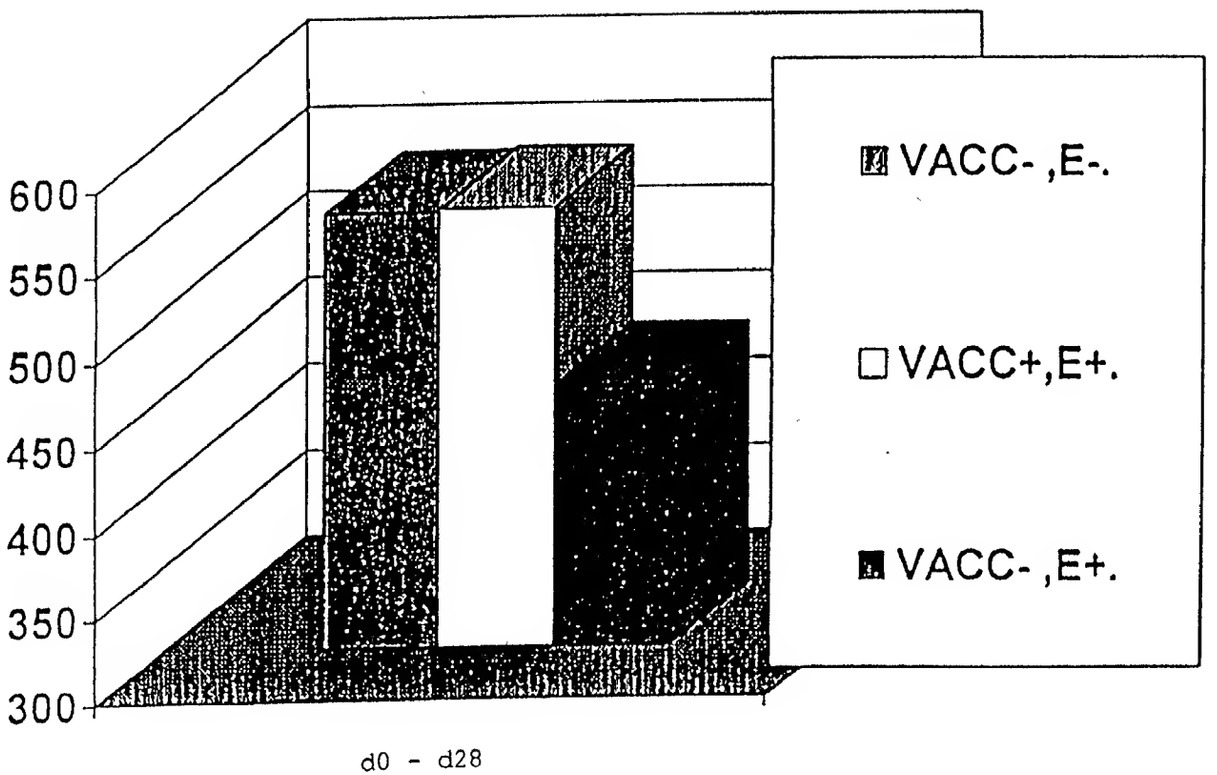


FIGURE 10

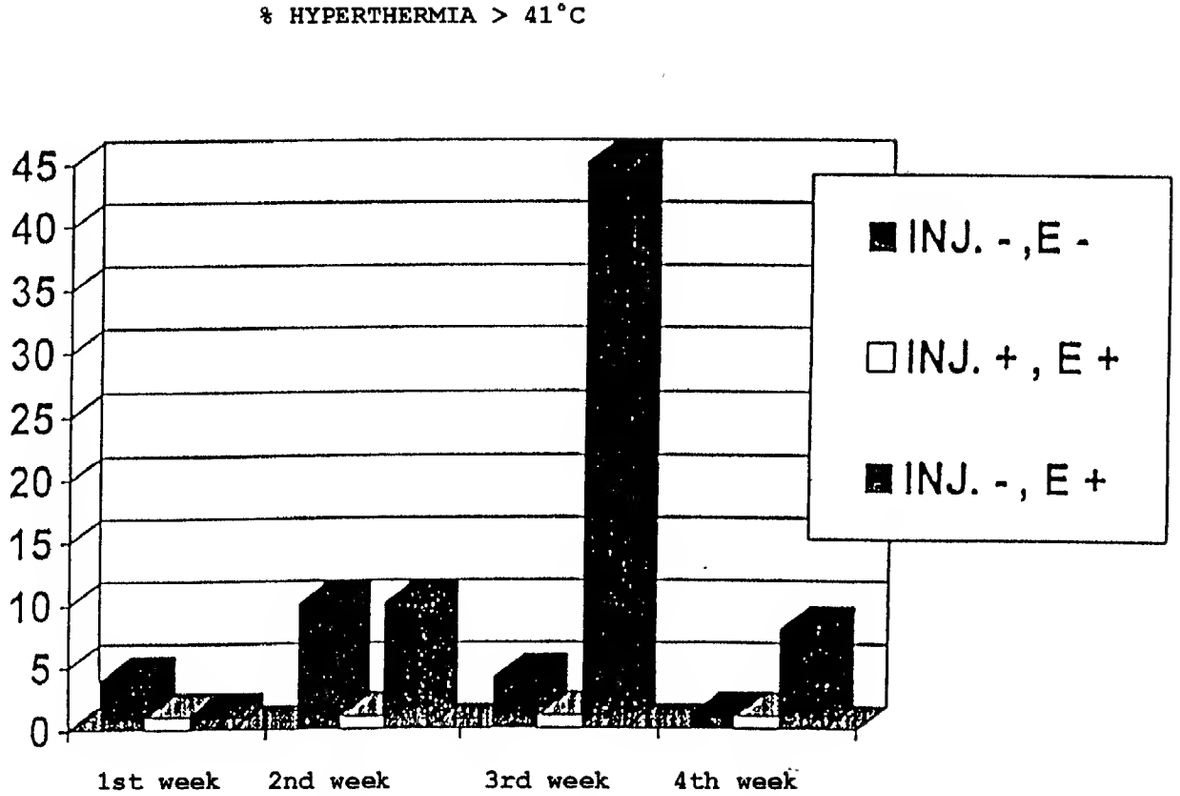

% HYPERTHERMIA > 41°C
45
40
35
30
25
20
15
10
5
0
1st week
2nd week
3rd week
4th week
INJ. - ,E -
INJ. + , E +
INJ. - , E +

FIGURE 11

Type B

spot No. 104 to 159

Type A

spot No. 160 to 215

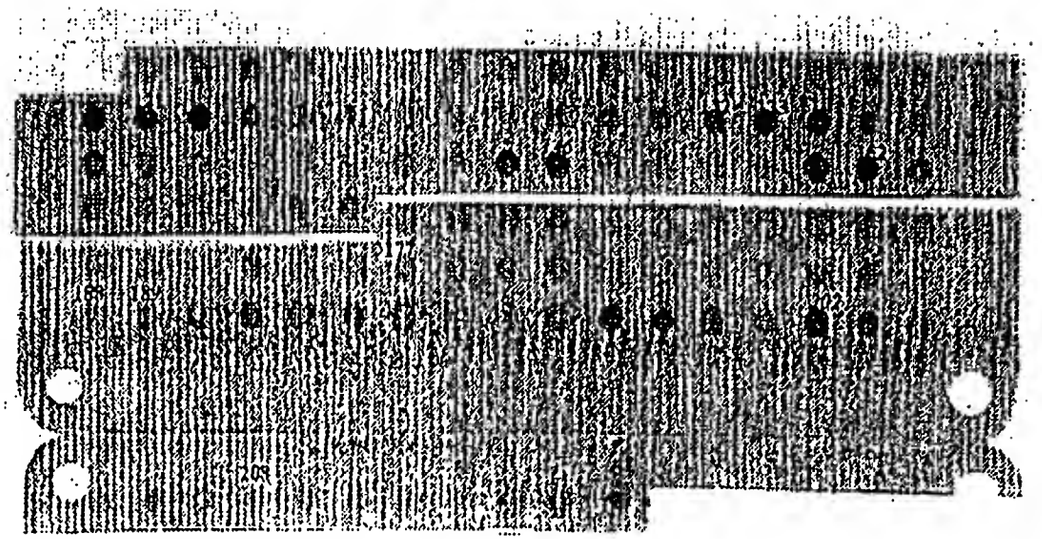
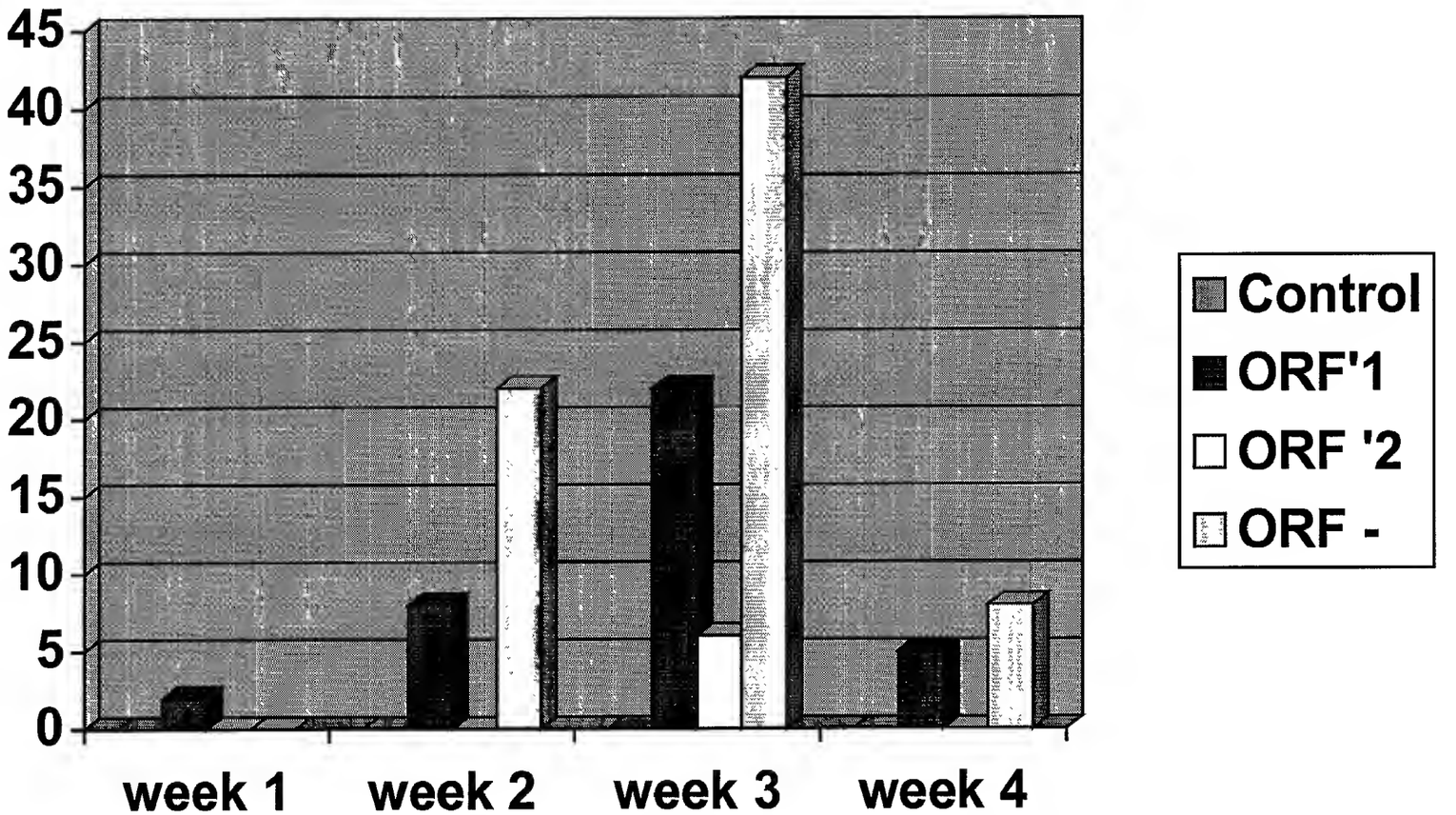


FIGURE 12

```
                                                                  50 51
pcvA  MTWPRRRYRR RRTRPRSHLG NILRRRPYLV HPAFRNRYRW RRKTGIFNSR LSREFVLTI. RGGHSQPSWN
pcvB  MTYPRRRYRR RRHRPRSHLG QILRRRPWLV HP..RHRYRW RRKNGIFNTR LSRTFGYTVK RTTVRTPSWA

          peptide 177              100 101         peptides 188 to 189
pcvA VNELRFNIGQ FLPPSGGTNP LPLPFQYYRI RKAKYEFYPR DPITSNQRGV GSTVVILDAN FVTPSTNLAY
pcvB VDMMRFNIND FLPPGGGSNP RSVPFEYYRI RKVKVEFWPC SPITQGDRGV GSSAVILDDN FVTKATALTY
          peptide 121                             peptides 132 to 133

          150 151                                                 peptide 208
pcvA DPYINYSSRH TIRQPFTYHS RYFTPKPELD QTIDWFQPNN KRNQLWLHLN THTNVEHTGL GYALQNATTA
pcvB DPYVNYSSRH TITQPFSYHS RYFTPKPVLD FTIDYFQPNN KRNQLWLRLQ TAGNVDHVGL GTAFENSIYD
                                                                   peptide 152

                           235
pcvA QNYVVRLTIY VQFREFILKD P.LNE
pcvB QEYNIRVTMY VQFREFNFKD PPLNP
```

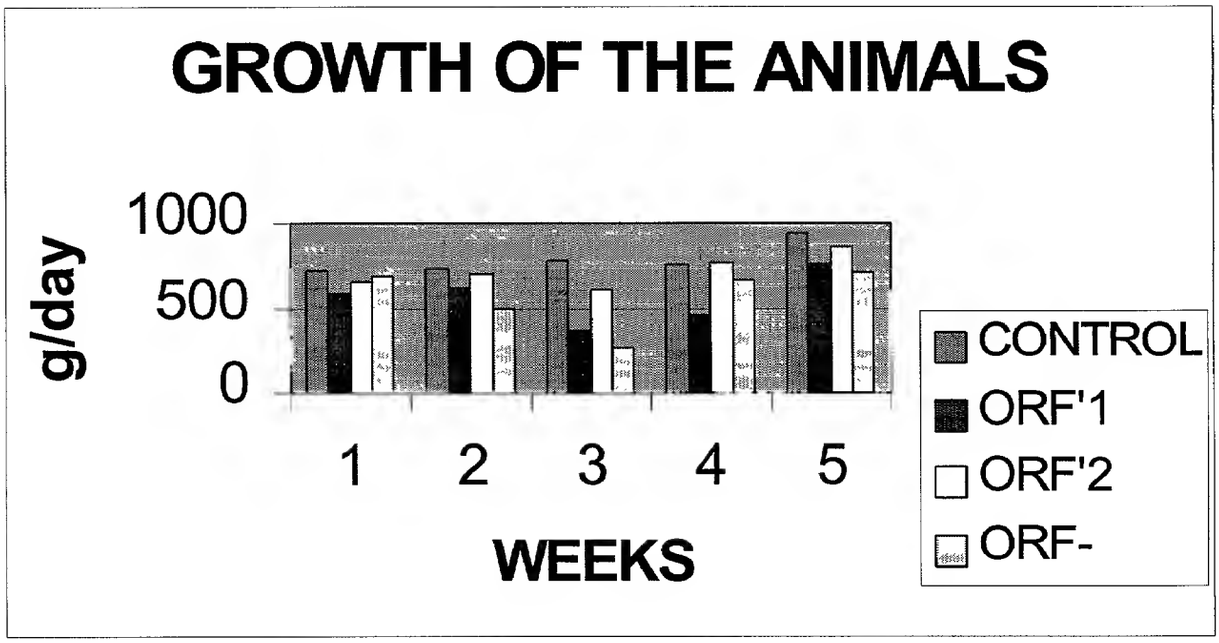
FIGURE 13

Figure 14



Percent hyperthermia (> 40.5°C), Control (not vaccinated and not challenged), ORF'1 (vaccinated and challenged), ORF'2 (vaccinated and challenged), ORF- (not vaccinated, challenged)

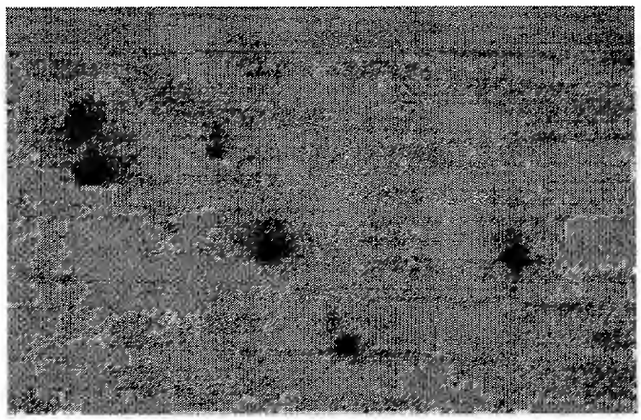
Figure 15



Control (not vaccinated and not challenged), ORF'1(vaccinated and challenged) ORF'2 (vaccinated and challenged), ORF- (not vaccinated, challenged)

Figure 16

Immunoperoxidase staining of PK15 cells at 24 h post-transfection with the pcDNA3/ORF'2 plasmid. Expression of PCV-B ORF'2 was confirmed by IPMA following incubation in the presence of the swine anti-PCV-B monospecific serum.



DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

CIRCOVIRUS SEQUENCES ASSOCIATED WITH PIGLET WEIGHT LOSS DISEASE (PWD)

(Attorney Docket No. 065691/0176)

the specification of which (check one)

___ is attached hereto.

__X__ was filed on 2/28/2000 as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code §119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
97/15396	France	December 5, 1997	Yes	

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number
	PCT/FR98/02634	December 4, 1998	

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith:

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I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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